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SPOROTRICHUM SPECIES: THEIR NITROGEN METABOLISM

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(WITH 1 FIGURE)

It has been recorded by Fulmer and Nelson that *Saccharomyces cerevisiae* can fix atmospheric nitrogen. This is quoted by Anderson (**1**) who is still uncertain whether yeasts can utilize nitrites and nitrates, the balance of opinion being that they are not utilized, especially under aerobic conditions. He states that ammonium salts, amines, amides and amino acids can act as nitrogen sources for yeasts, while proteins, proteoses and higher polypeptides are not utilized directly.

According to Smith (**7**) most of the common molds can make use of inorganic sources of all the elements except carbon, but a few are unable to use inorganic nitrogen. Others grow well on media containing ammonium salts but cannot utilize nitrogen from nitrates.

Nickerson (**5**) records that one of the first investigations on the nitrogen nutritional requirements of pathogenic fungi was that of Mosher *et al.* (**3**). Working with *Trichophyton mentagrophytes* they found that no growth occurred in the presence of inorganic nitrogen alone. In a synthetic medium it had to be supplied with amino acids. There was no evidence of the indispensability of any single amino acid, although there was very little growth without leucine, while asparagine and threonine promoted considerable growth. A varied assortment of amino acids was superior to any single amino acid or group of three or four. These

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findings were for the most part confirmed by Robbins and Ma (**6**). They found that with this same fungus inorganic nitrogen as NH_4NO_3 was almost completely unavailable. No single amino acid was indispensable and no single one was as good as a mixture. The near essentiality of leucine was not confirmed but it proved to be the best for promoting growth in the early stages. A most interesting finding was that a pleomorphic strain of *T. mentagrophytes* was able to utilize ammonium nitrate as the sole source of nitrogen.

Baker and Smith (**2**) found that *Coccidioides immitis* could not utilize nitrite as KNO_2 but nitrate as KNO_3 gave a fair growth, while good growths were obtained with NH_4Cl, urea, acetamide, asparagine, glycine, alanine, glutamic acid, tyrosine, cystine and peptone.

Negroni (**4**) confirmed these findings and reported that *Coccidioides immitis* could utilize peptone, asparagine, histidine, urea, ammonium sulphate, potassium nitrate and ammonium chloride.

No literature on the nitrogen metabolism of Sporotricha could be found.

The object of this investigation was to determine whether *Sporotrichum* species could utilize inorganic nitrogen, amides and amino acids, and whether any single amino acid was essential for growth.

TECHNIQUE

The principle of the method adopted was the growing of the fungus in various liquid media for four weeks, weighing the growth and comparing the weight of growth in the different media.

The formulae for the media used were as follows:

Medium 1. (*Basic medium*)

K_2HPO_4	1 gm.
KCl	0.5 gm.
$MgSO_4 \cdot 7H_2O$	0.5 gm.
$FeSO_4$	0.018 gm.
Maltose	4.0 gm.
Aq. dist.	1000 cc.

Medium 2. (*Casein control*)

Basic medium + 1% sodium caseinate (Difco).

Medium 3

Basic medium + 0.62% ammonium nitrate.

Medium 4

Basic medium + 0.62% urea.

Medium 5

Basic medium +	
Cystine hydrochloride	0.002%
dl-valine	0.08%
dl-threonine	0.06%
dl-isoleucine	0.08%
dl-phenylalanine	0.06%
l-arginine	0.06%
dl-methionine	0.06%
l-histidine	0.04%
l-tryptophane	0.04%
l-leucine	0.08%
l-asparagine	0.04%

Medium 6

Basic medium +	
Cystine hydrochloride	0.002%
dl-valine	0.08%
dl-threonine	0.06%
dl-isoleucine	0.08%
dl-phenylalanine	0.06%
l-arginine	0.06%
dl-methionine	0.06%
l-histidine	0.04%
l-tryptophane	0.04%
l-leucine	0.08%

Medium 7

Basic medium +	
Cystine hydrochloride	0.002%
dl-valine	0.08%
dl-threonine	0.06%
dl-isoleucine	0.08%
dl-phenylalanine	0.06%
l-arginine	0.06%
dl-methionine	0.06%
l-histidine	0.04%
l-tryptophane	0.04%

Medium 8

Basic medium +	
Cystine hydrochloride	0.002%
dl-valine	0.08%
dl-threonine	0.06%
dl-isoleucine	0.08%
dl-phenylalanine	0.06%
l-arginine	0.06%
dl-methionine	0.06%
l-histidine	0.04%

Medium 9

Basic medium +	
Cystine hydrochloride	0.002%
dl-valine	0.08%
dl-threonine	0.06%
dl-isoleucine	0.08%
dl-phenylalanine	0.06%
l-arginine	0.06%
dl-methionine	0.06%

Medium 10

Basic medium +	
Cystine hydrochloride	0.002%
dl-valine	0.08%
dl-threonine	0.06%
dl-isoleucine	0.08%
dl-phenylalanine	0.06%
l-arginine	0.06%

Medium 11

Basic medium +	
Cystine hydrochloride	0.002%
dl-valine	0.08%
dl-threonine	0.06%
dl-isoleucine	0.08%
dl-phenylalanine	0.06%

Medium 12

Basic medium +	
Cystine hydrochloride	0.002%
dl-valine	0.08%
dl-threonine	0.06%
dl-isoleucine	0.08%

Medium 13

Basic medium +	
Cystine hydrochloride	0.002%
dl-valine	0.08%
dl-threonine	0.06%

Medium 14

Basic medium +	
Cystine hydrochloride	0.002%
dl-valine	0.08%

Medium 15

Basic medium +	
Cystine hydrochloride	0.002%

Medium 16

Basic medium +	
dl-valine	0.08%

Medium 17

Basic medium +	
l-arginine	0.06%
dl-methionine	0.06%
l-histidine	0.04%

Medium 18

Basic medium +	
dl-phenylalanine	0.06%
dl-valine	0.08%
dl-threonine	0.06%

Medium 19

Basic medium +	
l-leucine	0.08%
l-tryptophane	0.04%
Cystine hydrochloride	0.002%

Medium 20

Basic medium +	
dl-isoleucine	0.08%
l-histidine	0.04%
dl-threonine	0.06%

Medium 21

Basic medium +	
l-tryptophane	0.04%
l-asparagine	0.04%
l-arginine	0.06%

The first amino acid medium (No. 5) contained eleven different amino acids. In each subsequent medium one amino acid was removed until finally only one remained (Nos. 15 and 16). In media Nos. 17 to 21 inclusive five different groups of three amino acids were used.

Each medium was made up in 500 cc. quantities. The appropriate ingredients were dissolved in approximately 500 cc. of distilled water, which was then filtered through several layers of gauze into a volumetric flask and the volume made up to 500 cc. with distilled water. The pH was adjusted to 6.8 and the medium dispensed in 50 cc. quantities into 100 cc. Erlenmeyer flasks, which were then autoclaved at 15 lb. pressure for 20 minutes.

Eight strains of *Sporotrichum* were investigated in the manner described, comprising:

No. 8. *S. asteroides,* Splendore, Westerdijk, Baarn. 1941.
No. 13. *S. schenckii,* Duke University, U. S. A. 1944.
No. 20. *Rhinocladium Beurmanni,* Langeron, Paris. 1934.
No. 21. *Rhinocladium equinum,* Sabouraud's collection. 1934.
No. 30. *S. acuminatum,* Tulane. 1945.
No. 32. *S. Beurmanni,* S. Africa. Isolated from a patient who contracted disease in a gold mine. 1947.
No. 61. *S. Beurmanni,* S. Africa. Isolated from timber in a gold mine. 1947.
No. 72. *S. tropicali,* Dr. Ghosh, India. 1947.

The cultures were maintained on Sabouraud's dextrose agar slopes.

One flask of each medium was inoculated with each of the eight strains. Cultures were left at room temperature for four weeks. At the end of this period each culture was filtered through Whatman No. 42, 7 cm. filter paper which had previously been weighed. Filtering was facilitated by the use of a Buchner funnel and slight suction. The filter papers were then placed in petri dishes and dried in vacuo over phosphorus pentoxide. They were then reweighed and the increase in weight recorded as the apparent weight of fungus growth. In the case of each medium a control uninoculated flask of medium was also filtered and the paper weighed. There was usually an increase of 1 or 2 mg. in weight

due to the presence of a slight precipitate. This was subtracted from the apparent weight of fungus growth for each strain. The final result was taken as the true weight of fungus growth.

The estimations of the growth in the basic medium (No. 1) and in the casein control (No. 2) were repeated three times and the figures shown are an average of these results.

The growth in Medium 2 containing sodium caseinate was taken as the maximum growth possible under the existing conditions. The weight of growth in the basic medium was subtracted from that in the caseinate medium and the difference taken as the maximum stimulation of growth by an excess of a readily available nitrogen source.

The weight of growth in the basic medium was subtracted from that in all other media. The weights of growth in all other media were subtracted from that in the casein control. From this it could be seen whether the addition of the various nitrogen sources produced any stimulation of growth and whether the stimulation, if any, was equal to or less than that of sodium caseinate.

Comparisons between one medium and another were also made.

The results are summarized in table 1. It will be seen that some growth, although very little, did occur in the basic medium. The growth was obvious to the naked eye. The only source of nitrogen in this medium was that from the atmosphere or from impurities in the chemicals. All the chemicals employed were the purest obtainable and manufactured by well-known reputable firms. It would appear, therefore, that these Sporotricha are capable of fixing atmospheric nitrogen.

The eight strains were grown in the basic medium for four weeks. A control uninoculated flask of medium was left standing for the same length of time. This was done in duplicate. At the end of four weeks the fungus growth was removed from one group of flasks by filtering in the usual way and the weight of growth ascertained. The control medium was also filtered. The growth from the second set of flasks was centrifuged, the deposit washed several times with distilled water and then transferred to small stoppered tubes, the weights of which were previously ascertained. After a final centrifuging as much of the supernatant fluid as pos-

TABLE 1

WEIGHTS OF FUNGUS GROWTH IN VARIOUS MEDIA AND COMPARISON OF THE WEIGHTS IN THE DIFFERENT MEDIA

Strain	Medium No. 1	Medium No. 2		Medium No. 3			Medium No. 4		
	Weight in mgms.	Weight in mgms.	Difference between weight and weight in Medium No. 1	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2
8	4.5	38	+33.5	13.5	+9	−24.5	11	+ 6.5	−27
13	5.5	49	+43.5	10	+4.5	−39	16	+10.5	−33
20	5.5	46	+40.5	9	+3.5	−37	10	+ 4.5	−36
21	5.5	38	+32.5	8.5	+3	−29.5	16	+10.5	−22
30	8.5	50	+41.5	12.5	+4	−37.5	10.5	+ 2	−39.5
32	6	43	+37	9	+3	−34	7.5	+ 1.5	−35.5
61	4.5	42	+37.5	10.5	+6	−31.5	10.5	+ 6	−31.5
72	5	68	+63	8	+3	−60	12	+ 7	−56

Strain	Medium No. 5			Medium No. 6				Medium No. 7			
	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 5	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 6
8	31	+26.5	− 7	41	+36.5	+ 3	+10	31.5	+27	− 6.5	− 9.5
13	20	+14.5	−29	31.5	+26	−17.5	+11.5	24.5	+19	−24.5	− 7
20	17.5	+12	−28.5	21.5	+16	−24.5	+ 4	27	+21.5	−19	+ 5.5
21	17.5	+12	−20.5	38	+32.5	0	+20.5	34.5	+29	− 3.5	− 3.5
30	16.5	+ 8	−33.5	59	+50.5	+ 9	+42.5	45	+36.5	− 5	−14
32	17	+11	−26	56.5	+50.5	+13.5	+39.5	35	+29	− 8	−21.5
61	20	+15.5	−22	60.5	+56	+18.5	+40.5	30	+25.5	−12	−30.5
72	18.5	+13.5	−49.5	48	+43	−20	+29.5	30	+25	−38	−18

Strain	Medium No. 8				Medium No. 9			
	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 7	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 8
8	14.5	+10	−23.5	−17	9.5	+ 5	−28.5	−5
13	17.5	+12	−31.5	− 7	23.5	+18	−25.5	+6
20	22	+16.5	−24	− 5	17	+11.5	−29	−5
21	22.5	+17	−15.5	−12	21	+15.5	−17	−1.5
30	18.5	+10	−31.5	−26.5	16	+ 7.5	−34	−2.5
32	22	+16	−21	−13	15.5	+ 9.5	−27.5	−6.5
61	20.5	+16	−21.5	− 9.5	18.5	+14	−23.5	−2
72	24.5	+19.5	−43.5	− 5.5	20.5	+15	−47.5	−4

TABLE 1—*Continued*

Strain	Medium No. 10				Medium No. 11			
	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 9	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 10
8	7	+ 2.5	−31	− 2.5	6	+1.5	−32	−1
13	11	+ 5.5	−38	−12.5	12	+6.5	−37	+1
20	11.5	+ 6	−34.5	− 5.5	9	+3.5	−37	−2.5
21	13.5	+ 8	−24.5	− 7.5	13.5	+8	−24.5	0
30	12	+ 3.5	−38	− 4	11	+2.5	−39	−1
32	19.5	+13.5	−23.5	+ 4	14.5	+8.5	−28.5	−5
61	16	+11.5	−26	− 2.5	13.5	+9	−28.5	−2.5
72	16	+11	−52	− 4.5	9	+4	−59	−7

Strain	Medium No. 12				Medium No. 13			
	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 11	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 12
8	6	+ 1.5	−32	0	8.5	+ 4	−29.5	+2.5
13	10.5	+ 5	−38.5	−1.5	10	+ 4.5	−39	−0.5
20	10.5	+ 5	−35.5	+1.5	9.5	+ 4	−36.5	−1
21	9.5	+ 4	−28.5	−1	8.5	+ 3	−29.5	−1
30	12.5	+ 4	−37.5	+1.5	10	+ 1.5	−40	−2.5
32	14	+ 8	−29	−0.5	11.5	+ 5.5	−31.5	−2.5
61	16.5	+11.5	−25.5	+3	15	+10.5	−27	−1.5
72	16	+11	−52	+7	14	+ 9	−54	−2

Strain	Medium No. 14				Medium No. 15			
	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 13	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 14
8	6.5	+2	−31.5	−2	6	+1.5	−32	−0.5
13	8	+2.5	−41	−2	6.5	+1	−42.5	−1.5
20	8.5	+3	−37.5	−1	7	+1.5	−39	−1.5
21	9.5	+4	−28.5	+1	6	+0.5	−32	−3.5
30	10	+1.5	−40	0	9	+0.5	−41	−1
32	11.5	+5.5	−31.5	0	10	+4	−33	−1.5
61	9	+4.5	−33	−6	9.5	+5	−32.5	+0.5
72	8	+3	−60	−6	6.5	+1.5	−61.5	−1.5

TABLE 1—*Continued*

Strain	Medium No. 16				Medium No. 17			
	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 14	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 13
8	8.5	+4	−29.5	+2	19.5	+15	−18.5	+11
13	10	+4.5	−39	+2	24	+18.5	−25	+14
20	7.5	+2	−38.5	−1	20.5	+15	−25.5	+11
21	9	+3.5	−29	−0.5	15.5	+10	−22.5	+ 7
30	9	+0.5	−41	−1	28	+19.5	−22	+18
32	6	0	−37	−5.5	21.5	+15.5	−21.5	+10
61	8.5	+4	−33.5	−0.5	19.5	+15	−22.5	+ 4.5
72	7	+2	−61	−1	23	+18	−45	+ 9

Strain	Medium No. 18				Medium No. 19			
	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 13	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 13
8	10.5	+6	−27.5	+2	9.5	+5	−28.5	+1
13	10	+4.5	−39	0	8.5	+3	−40.5	−1.5
20	11	+5.5	−35	+1.5	10	+4.5	−36	+0.5
21	8	+2.5	−30	−0.5	11	+5.5	−27	+2.5
30	10.5	+2	−39.5	+0.5	9.5	+1	−40.5	−0.5
32	13	+7	−30	+1.5	11	+5	−32	−0.5
61	14	+9.5	−28	−1	11	+6.5	−31	−4
72	12	+7	−56	−2	12	+7	−56	−2

Strain	Medium No. 20				Medium No. 21			
	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 13	Weight in mgms.	Difference between weight and weight in Medium No. 1	Difference between weight and weight in Medium No. 2	Difference between weight and weight in Medium No. 13
8	12	+ 7.5	−26	+3.5	9	+4.5	−29	+0.5
13	17	+11.5	−32	+7	12	+6.5	−37	+2
20	13	+ 7.5	−33	+3.5	7.5	+2	−38.5	−2
21	18	+12.5	−20	+9.5	8.5	+3	−29.5	0
30	12.5	+ 4	−37.5	+2.5	10	+1.5	−40	0
32	16.5	+10.5	−26.5	+5	10.5	+4.5	−32.5	−1
61	19.5	+15	−22.5	+4.5	10.5	+6	−31.5	−4.5
72	14.5	+ 9.5	−53.5	+0.5	9	+4	−59	−5

sible was removed. The tubes without their stoppers were then placed in a desiccator containing phosphorus pentoxide, which was then evacuated. When the fungus deposit was completely dry, the stoppers were quickly replaced and the tubes reweighed. The difference in weight was the weight of fungus growth. The filtrates of the first series of flasks and the dried fungus of the second series were then subjected to the micro-Kjeldahl technique for the estimation of total nitrogen by Dr. C. G. Anderson.

In order to determine the weight of actual growth of the fungus it was necessary to subtract the weight of the inoculum. This was estimated by taking twenty portions of the fungus from the stock culture, each of a size approximating the usual inoculum. These were placed on a coverglass of known weight and dried in vacuo over phosphorus pentoxide. It was then reweighed, the weight of the coverglass subtracted and the difference divided by 20.

The weight of the inoculum was subtracted from the weight of growth. The amount of nitrogen in this weight of fungus was then calculated.

The results are summarized in table 2. It will be seen that a trace of nitrogenous material (71 μg.) was present in the original medium. After the fungus had grown for four weeks, small quantities of this nitrogen had been removed from the medium (13 to 64.5 μg.). It can be seen, however, that in the case of six strains the fungus growth contained considerably more nitrogen than that removed from the medium in which it was grown. This excess must have been derived from the atmosphere. Bearing in mind the extremely small quantities being estimated and possibility of experimental error, it nevertheless would appear that these Sporotricha are capable of fixing atmospheric nitrogen.

Referring to table 1 it can be seen that both ammonium nitrate and urea are capable of supporting growth, the latter being slightly more effective.

A mixture of ten amino acids (Medium No. 6) is as good a source of nitrogen as is sodium caseinate. It would appear that asparagine in the concentration used (0.04 per cent) retards growth as its removal in Medium No. 6 and No. 7 gave better growth. This finding was confirmed on repeating the experiment. As the number of amino acids in the medium is reduced so is there

TABLE 2

MICRO-KJELDAHL ESTIMATIONS OF THE NITROGEN CONTENT OF THE FUNGUS GROWTHS AND THEIR RESPECTIVE MEDIA

Strain	Weight of growth after four weeks in μg.	Percentage of nitrogen in dry fungus	Percentage of protein in dry fungus	Total nitrogen content of fungus in μg. (a)	Nitrogen content of inoculum (wt. 775 g.) in μg. (b)	Nitrogen content of actual fungus growth in μg. (c = a–b)	Nitrogen content of filtered medium in μg.	Nitrogen removed from medium by fungus in μg. (d)	Nitrogen which could only have been derived from atmosphere in μg. (c–d)
Control							71		
8	3,500	2.7	16.9	94.5	21	73.5	40.5	30.5	43
13	5,000	1.4	8.7	70	11	59	58	13	46
20	5,000	1.9	11.9	95	15	80	37	34	46
21	4,500	1.5	9.4	67.5	11.5	56	6.5	64.5	0
30	9,500	1.5	9.4	142.5	11.5	131	27	44	87
32	6,000	1.6	10.0	96	12.5	83.5	44	27	56.5
61	3,000	2.0	12.5	60	15.5	44.5	44	27	17.5
72	3,000	1.7	10.6	51	13	38	20	51	0

a gradual retardation of the growth. The removal of certain amino acids causes a greater retardation than others, *e.g.* leucine, tryptophane, histidine and methionine appear to be more important for growth than arginine, phenylalanine, isoleucine, threonine, valine and cystine. The presence of only one amino acid in the medium produced a very slight improvement in growth over that in the basic medium. Six different combinations of three amino acids in each were compared (Medium No. 13, 17, 18, 19, 20 and 21). Of these, four (No. 13, 18, 19 and 21) were more or less the same, No. 20 gave slightly better growth and No. 17 (arginine, methionine and histidine) was the best, being almost equivalent to

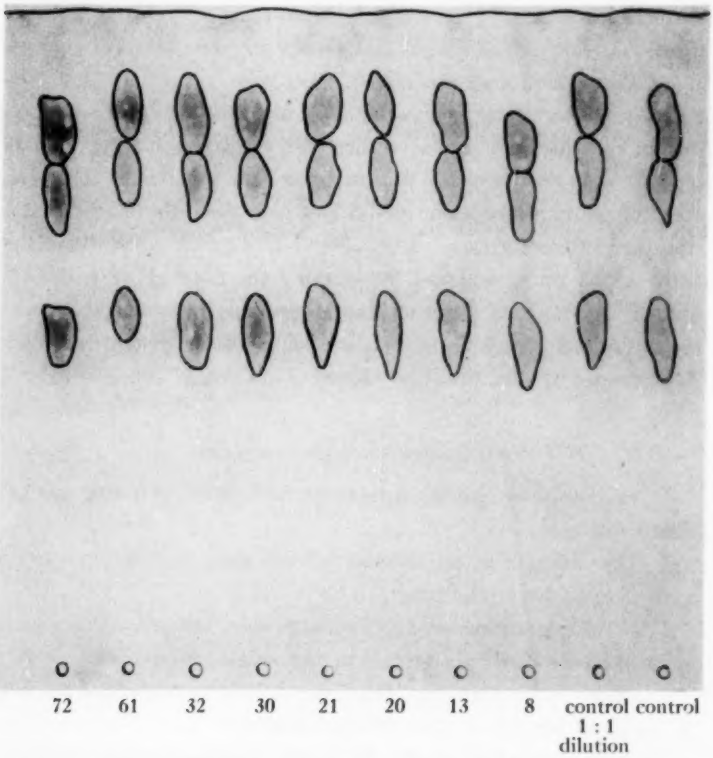

FIG. 1. Paper chromatographic analysis of amino acid Medium No. 20, uninoculated and after four weeks growth of the different strains.

Medium No. 8, which contains these three amino acids and five others.

These findings agree well with those of Mosher *et al.* (**3**) and those of Robbins and Ma (**6**) that no single amino acid is indispensable and that a varied assortment of amino acids is superior to any single amino acid or group of three or four.

In order to determine whether any amino acid had been completely or partially removed from the medium or whether any new amino acid had appeared as a result of the metabolism of the fungus, paper chromatographic analyses were attempted. In Medium No. 13, 18, 19, 20 and 21 the amino acids were arranged in groups of three, so that their R_f values would be as far apart as possible in order to give adequate separation with a single directional run. After the fungus had grown for four weeks, the media were filtered and volumes of 0.025 cc. were applied to the filter paper to form spots approximately 5 mm. in diameter. In the case of each medium employed the control uninoculated medium as well as a 1 : 1 dilution of this medium was applied to the same sheet of paper as the eight media in which the different strains of Sporotricha were grown. Phenol was used as the solvent and the amino acids were displayed by spraying the filter paper with ninhydrin. In no case was there any detectable reduction of any of the amino acids and no new amino acids could be demonstrated. An example of the results obtained is shown in the photograph (Fig. 1).

CONCLUSIONS AND SUMMARY

1. *Sporotrichum* species appear to be capable of fixing atmospheric nitrogen.

2. The nitrogen of ammonium nitrate, urea and various amino acids is available to the fungi.

3. No single amino acid is indispensable, but a varied assortment of amino acids is superior to any single amino acid.

4. There is no apparent difference in the nitrogen metabolism of the eight strains of *Sporotrichum* studied.

My thanks are due to Dr. W. I. M. Holman for his continual interest and advice, to Dr. C. G. Anderson for carrying out the

micro-Kjeldahl determinations, to Mr. H. D. Barnes for his assistance with the chromatography, and to Mr. F. A. Brandt for the photograph.

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NUTRIENT REQUIREMENTS FOR TWO SPECIES OF AQUATIC HYPHOMYCETES [1]

FRANCIS V. RANZONI

(WITH 4 FIGURES)

There is contained within the form-family Moniliaceae an interesting group of fresh-water aquatic fungi, consisting of 17 genera and 28 species (**2**, **3**, **5**), that grow in the vascular systems of submerged and decaying angiosperm debris. The spores are normally formed and released beneath the surface of the water. Although many of these fungi have been successfully grown in pure culture on corn meal agar, oat meal agar and other natural solid media (**2**), no attempts have been made to otherwise define the growth requirements of any of these organisms. As part of a general taxonomic and morphological investigation (**5**), two species in the genus *Anguillospora*, closely related on morphological grounds, but differing sharply in cultural characteristics and in the method of spore release, were subjected to a study of their nutrient requirements to determine whether the morphological differences were reflected in nutritional differences.

Anguillospora longissima (Sacc. & Syd.) Ingold (**2**) is characterized by a greenish-black mycelium on malt agar and multicellular, sigmoid or falcate scolecospores, 150–350 μ in length, that are released by the break-down of a specialized separating cell. *Anguillospora gigantea* Ranzoni (**5**), on the other hand, is characterized by a reddish-purple mycelium on malt agar and multicellular, sigmoid or falcate scolecospores, 150–750 μ in length, that are released by a wall disarticulation and rounding-off process.

Both organisms were originally obtained by single-spore isolations and subsequently maintained in stock-culture on 2 per cent

[1] I wish to express my deep appreciation to Professor L. Machlis for his enthusiastic and continued interest and his help throughout the course of this investigation.

malt agar slants (initial pH of 5.5) on which they could be kept for several months without transfer. Spores for the inoculation of liquid cultures were readily obtained by placing narrow strips of the agar colony in water for 18 to 24 hours. After release the spores were easily picked up by means of Pasteur pipettes.

The ability to grow in the various media was measured in terms of the dry weight produced. The organisms were grown in 75 ml. of liquid culture medium in Erlenmeyer flasks in triplicate and the values reported are the averages.

DETERMINATION OF THE EXPERIMENTAL CONDITIONS

Both species were found to grow well in a medium composed of 1.0 gm. glucose, 0.1 gm. KH_2PO_4, 0.02 gm. $MgSO_4 \cdot 7H_2O$, 0.002 gm. $FeCl_3 \cdot 6H_2O$, and 0.3 gm. Difco Yeast Extract per 100 ml. of distilled water (Medium I).

Preliminary experiments indicated that optimum growth of these species was dependent upon adequate aeration (TABLE I). It is

TABLE I

EFFECT OF AERATION ON THE DRY WEIGHTS OF MYCELIA OF *A. longissima* AND *A. gigantea* IN MEDIUM I ADJUSTED TO pH 6.5 AND INCUBATED AT 28° C UNDER THE SPECIFIED CONDITIONS FOR THREE WEEKS

Conditions	Dry weight in mgms.	
	A. longissima	*A. gigantea*
125 ml. standing flasks	37.9	42.2
125 ml. shaking flasks	137.1	99.0
250 ml. standing flasks	160.3	136.1
250 ml. shaking flasks	258.2	232.7

apparent that increasing the size of the flask while holding the volume of medium constant greatly increased the final dry weight. When, in addition, the aeration was augmented by agitation obtained by means of a mechanical horizontal shaker there was a further substantial increase in dry weight. Since the only shaker available for agitation was in a 28° C room, subsequent experiments were done at this temperature in either 250 ml. flasks or 125 ml. flasks. An early experiment done with standing (non-shaken) 125 ml. flasks indicated that the organisms have a relatively broad temperature tolerance. Under these conditions *A.*

longissima showed a preference for a lower temperature than 28° C (FIG. 1). This could not be checked, however, under the optimum conditions of aeration.

The optimum initial pH for growth at 28° C with shaking was determined to be between 6.5 and 6.8. After three weeks of

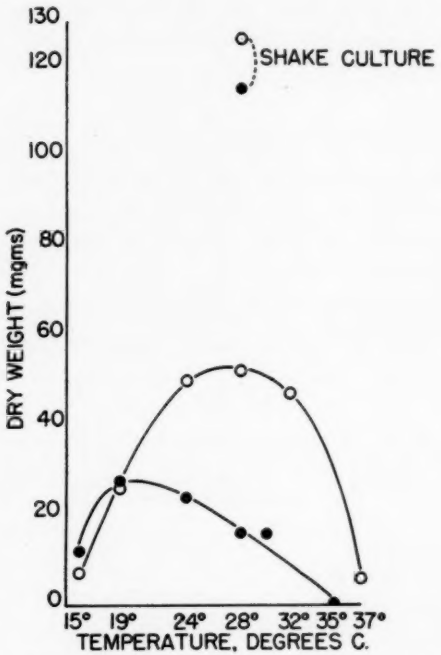

FIG. 1 (left). Effect of temperature on the dry weights of *A. longissima* (black dot) and *A. gigantea* (white dot) grown in 75 ml. of yeast extract medium in 125 ml. flasks as non-shaken cultures and incubated at the indicated temperatures. Note, for comparison, the dry weights for the shake cultures grown in 125 ml. flasks containing the same medium and incubated at 28° C.

growth in media at initial pH values of 4 to 7.5, negligible changes in pH were observed except where *A. gigantea* was started at pH values of 6 to 7.5. Here an appreciable drop in pH was found, indicative, perhaps, of acid formation. This aspect of the problem was not studied, however.

In the preceding experiments dry weights were taken after three weeks of growth. While it seemed clear that this was an adequate growth period, it was not at all certain that maximum growth was obtained during this time. This was tested and the results (FIG. 2)

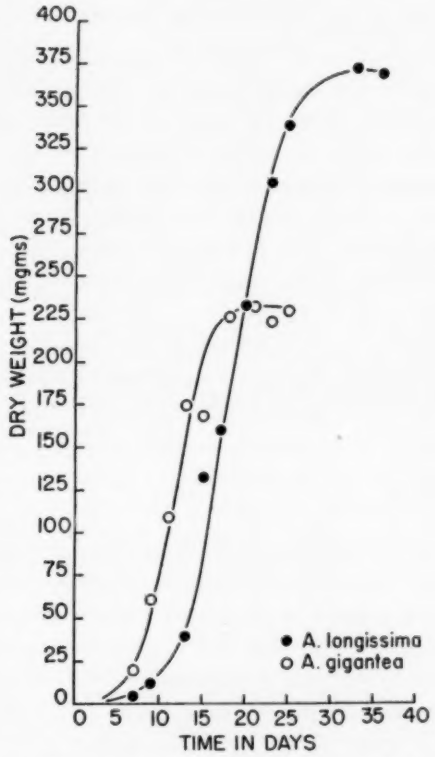

FIG. 2 (right). Effect of time on the dry weights of *A. longissima* and *A. gigantea* grown in 75 ml. of yeast extract medium in 250 ml. flasks on a shaker and incubated at 28° C.

indicated that *A. gigantea* requires 21 days and *A. longissima* 29 days to attain maximum dry weight.

On the basis of the preliminary experiments, the conditions adopted for the determination of the specific nutrient requirements of these fungi were as follows: growth for three weeks, unless

visual inspection indicated that more time was necessary, in 75 ml. of medium at an initial pH of 6.5 and at a temperature of 28° C. Aeration was supplied by the use of a horizontal shaker that caused a vigorous swirling of the liquid medium.

DETERMINATION OF THE NUTRIENT REQUIREMENTS

In the preliminary experiments vitamins and amino acids of unknown identity and amount, organic nitrogen and other constituents were supplied to the fungi by the yeast extract in the medium. When the yeast extract is replaced by 1 gm. KNO_3 per 100 ml. of medium (Medium II) no growth occurred (TABLE II). When, however, 1.3 ml. of 10 per cent vitamin-free casein hydrolysate (acid hydrolized) and 12.6 gamma each of folic acid, riboflavin, nicotinamide, biotin, p-aminobenzoic acid, pyridoxin, calcium pantothenate, and thiamin hydrochloride are added per 100 ml. of medium, good growth occurred (Medium III).[2] Growth occurred in the absence of the casein hydrolysate only when the vitamins were added (Medium IV). When the medium contained only the casein hydrolysate in addition to the salts and glucose no growth occurred (Medium V) (TABLE III). It is clear that these organisms will grow in an inorganic medium with KNO_3 as a source of nitrogen provided one or more vitamins are supplied. Though, as will be shown later (FIG. 4), substantially increased dry weights of mycelium could be obtained using nitrate as a source of nitrogen if the growth period is prolonged, the addition

TABLE II

EFFECT OF MEDIUM I AND MEDIUM II ON THE DRY WEIGHTS OF *A. longissima* AND *A. gigantea* GROWN IN 125 ML. FLASKS FOR THREE WEEKS

Media	Dry weights in mgms. *A. longissima*	*A. gigantea*
Medium I	158.2	127.0
Medium II	4.2	2.9

[2] The sources of these materials are: vitamin-free casein hydrolysate (Nutritional Biochemical Corp.); folic acid, riboflavin, nicotinamide, pyridoxin, calcium pantothenate and thiamin hydrochloride (all Nutritional Biochemical Corp.); biotin (Hoffmann-la Roche Inc.); p-aminobenzoic acid (Eastman Kodak Co.).

TABLE III

EFFECT OF MEDIUM I (CONTAINING YEAST EXTRACT), MEDIUM III (CONTAINING VITAMINS AND CASEIN HYDROLYSATE), MEDIUM IV (CONTAINING VITAMINS AND NITRATE) AND MEDIUM V (CONTAINING CASEIN HYDROLYSATE AND NO VITAMINS) ON THE DRY WEIGHTS OF *A. longissima* AND *A. gigantea* GROWN IN 250 ML. FLASKS FOR THREE WEEKS

Media	Dry weights in mgms. *A. longissima*	*A. gigantea*
Medium I	240.6	262.2
Medium III	227.4	254.9
Medium IV	76.7	83.7
Medium V	6.8	6.0

of casein hydrolysate to the medium was continued since higher dry weights could be obtained in a shorter growing period.

The particular vitamin required was determined by a series of deficiency cultures. The media used consisted of the usual salts, nitrate, casein hydrolysate and a series of vitamin combinations in which one vitamin was omitted and the other seven retained. As shown in Table IV, no growth occurred when thiamin was the vitamin omitted. Three successive transfers from the original media to freshly prepared media for each of the vitamin combinations yielded comparable dry weights in all cases. When the concentration of thiamin was varied from zero to 100 gamma per 75 ml. of medium it was found that the increase in concentration of thiamin resulted in proportional increases in the dry weights of

TABLE IV

EFFECT OF VITAMIN DEFICIENCIES ON THE DRY WEIGHTS OF *A. longissima* AND *A. gigantea* GROWN IN A MEDIUM CONSISTING OF GLUCOSE, MINERAL SALTS, NITRATE, CASEIN HYDROLYSATE AND THE INDICATED VITAMIN COMBINATIONS (EACH VITAMIN AT A CONCENTRATION OF 12.6 GAMMA PER 100 ML. OF MEDIUM) IN 250 ML. FLASKS FOR FOUR WEEKS

Vitamin combinations	Dry weights in mgms. *A. longissima*	*A. gigantea*
All but folic acid	295.8	324.4
All but riboflavin	280.6	347.9
All but nicotinamide	312.7	331.9
All but biotin	238.8	339.7
All but p-aminobenzoic acid	289.3	348.2
All but pyridoxin	283.8	324.7
All but calcium pantothenate	260.8	324.4
All but thiamin hydrochloride	6.7	3.2
No vitamins	2.9	2.2
All vitamins	311.2	324.8

both species (FIG. 3). Although maximum growth of both species was obtained when the concentration of thiamin was between 50 and 100 gamma, the response to increasing thiamin concentrations began to level off at 5–10 gamma for *A. gigantea* and at about 10 gamma for *A. longissima* (FIG. 3). Though high, these amounts of thiamin are in line with the requirements of other fungi for this vitamin (**6, 7**).

A similar response to thiamin concentration was found when nitrate was supplied as the only source of nitrogen. Of course, the ultimate dry weight produced was less than it would have been had the casein hydrolysate also been present and takes longer to be produced—in this instance five weeks instead of the usual three (FIG. 4).

CARBOHYDRATE UTILIZATION

Both species were found in nature growing in the vascular systems of angiosperm debris and, in addition, it was found that they grew well in culture with malt extract, prune agar and glucose as carbon sources. This indicated a broad latitude in carbohydrate requirements for these fungi. The organisms were grown in media composed of mineral salts, nitrate, casein hydrolysate, thiamin (10 gamma per 75 ml. of medium) and one of eleven carbohydrates.[3] Excellent growth was obtained for both species on all carbohydrates supplied except cellulose, pectin and arabinose (TABLE V). A difference in the enzyme systems of the two fungi may be indicated by the difference in their ability to utilize arabinose.

An unexpected result was the absolute absence of growth by both species in the cellulose cultures. In view of the natural substrata and the poor growth on pectin this was surprising. Either the fungi are lignin decomposers or else the absence of growth in the cellulose culture was due more to its physical nature than to anything else (lignin was not one of the carbohydrates tested as lignin prepared by the method of Brauns (**1**), was not available and its extraction is a lengthy and complicated process). Since,

[3] The sources of the carbohydrates tested are as follows: starch (Phanstiehl Chem. Co.); sucrose (Mallinckrodt Chem. Works); glucose (Merck and Co.); cellobiose, maltose, galactose, mannose, xylose, arabinose, pectin N.F. (all Nutritional Biochemical Corp.); cellulose extracted by the method of McBeth and Scales (**4**).

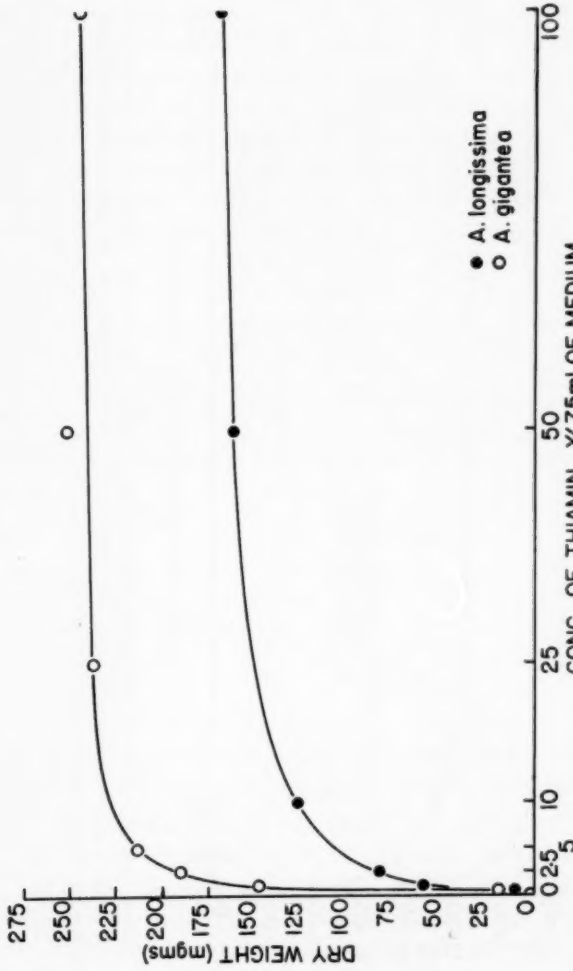

FIG. 3 (upper). Effect of increasing concentrations of thiamin on the dry weights of *A. gigantea* and *A. longissima* grown in 75 ml. of a medium containing, in addition to the thiamin, mineral salts, glucose, nitrate and casein hydrolysate in 250 ml. flasks on a shaker and incubated at 28° C.

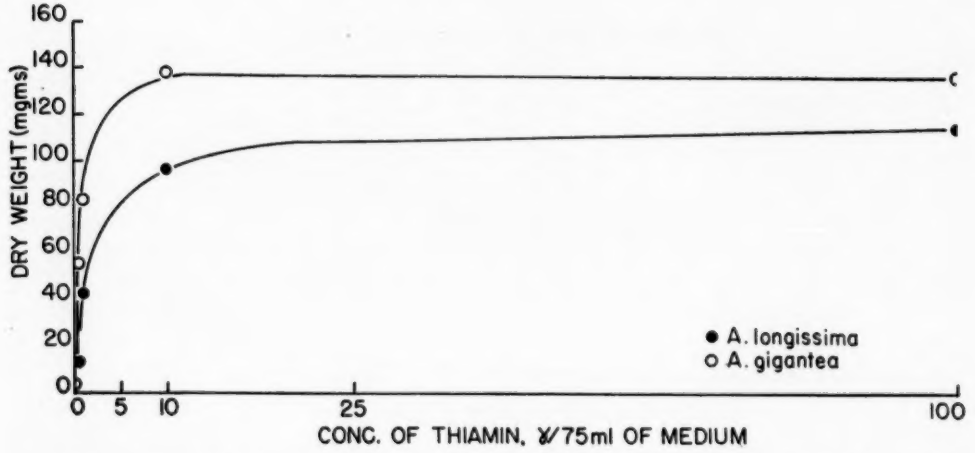

FIG. 4 (lower). Effect of increasing concentrations of thiamin on the dry weights of *A. gigantea* and *A. longissima* grown in 75 ml. of a medium that contains nitrate as the only nitrogen source in 250 ml. flasks on a shaker and incubated at 28° C.

however, the culture medium containing the extracted cellulose in suspension had a thick, syrupy consistency, it was possible that the physical nature of the medium may have interfered with the gaseous exchanges even though the cultures were grown on a shaker. Two sets of media composed of mineral salts, nitrate, casein hydrolysate and thiamin, one set containing glucose and the other cellulose "solution," were solidified with previously sterilized 2.5 per cent purified agar (**6**). Petri dish cultures thus prepared were inoculated with spores and allowed to grow for four weeks.

The cultures of *A. gigantea* were perfectly normal in appearance in both cellulose and glucose cultures. For the cellulose cultures

TABLE V

EFFECT OF MEDIA COMPOSED OF MINERAL SALTS, NITRATE, CASEIN HYDROLYSATE, THIAMIN HYDROCHLORIDE (12.6 GAMMA PER 100 ML. OF MEDIUM) AND THE INDICATED CARBOHYDRATES ON THE DRY WEIGHTS OF *A. longissima* AND *A. gigantea* GROWN IN 250 ML. FLASKS FOR FOUR WEEKS

	Dry weights in mgms.	
Carbohydrates supplied	*A. longissima*	*A. gigantea*
Starch	139.2	261.7
Cellulose	none	none
Cellobiose	298.7	283.4
Sucrose	200.8	292.7
Maltose	234.1	332.7
Glucose	279.5	305.1
Galactose	203.7	325.8
Mannose	304.7	303.2
Xylose	282.2	309.6
Arabinose	65.2	287.4
Pectin	84.8	77.2
None	8.3	5.0

of *A. longissima*, however, there was a striking deviation in pigmentation from that observed in the glucose and malt agar cultures; the pigment in cellulose cultures was a light reddish-purple, similar to the pigmentation of *A. gigantea*, instead of being a greenish-black as is the case when *A. longissima* is grown in glucose and malt agar culture. For both species on cellulose agar there were observed peripheral clearing effects. Microscopic examination of these cultures showed no indication that the organisms were starved—the hyphae were normal in appearance, diameter and branching. Perfectly normal spores were obtained, identical with those found in nature, when slices of the cellulose

and glucose cultures were placed in water. When the casein hydrolysate was omitted from the media spores were also formed but not in such great abundance.

DISCUSSION

The investigation reported here was begun in an attempt to add physiological characters to the standard morphological and developmental characters in the classification of the aquatic Fungi Imperfecti. It is not surprising, however, that the two species studied, *Anguillospora longissima* and *A. gigantea*, require the same nutrients, since they, together with all the other members of this group, occur in mixed populations on the common submerged, angiosperm vascular substratum. In fact, it is to be expected that the remaining species will also be heterotrophic for thiamin.

Although no qualitative differences in nutrition were found there were a number of minor quantitative differences, such as temperature, growth rate and pH, that should be expected to exist between species that appear to be as closely related as these.

SUMMARY

1. *Anguillospora longissima* and *A. gigantea* were found to grow best at 19° C and 28° C, respectively, in standing culture and both at 28° C in shake culture, between pH 6.5 and pH 6.7, and to attain maximum dry weight in between three and four weeks in a liquid medium composed of glucose, mineral salts and yeast extract.

2. The two species were found to be heterotrophic for thiamin and a carbohydrate source only. They are able to utilize nitrate as a source of nitrogen and sulphate as a source of sulphur. The usual mineral salts are presumably required as well.

3. The organisms can satisfactorily utilize a wide range of carbohydrates that includes starch, cellulose, cellobiose, sucrose, maltose, glucose, galactose, mannose, and xylose. *Anguillospora gigantea* was also found to grow well on arabinose and made only poor growth on pectin. *A. longissima*, on the other hand, did not grow well either on arabinose or on pectin.

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GROWTH OF SAPROLEGNIACEAE IN SYNTHETIC MEDIA. I. INORGANIC NUTRITION

HELEN SIMPSON REISCHER

Studies of the inorganic growth requirements of suitable fungi are a necessary preliminary to identification of the roles of the required elements at the surface of and inside the fungal cell. The elimination of inorganic materials as limiting factors in growth makes possible more precise study of other aspects of nutrition as well as such widely interesting physiological problems as those of morphogenesis or the ecologic niches filled by such fungi as the Saprolegniaceae which are nearly ubiquitous in soil and water.

The inorganic nutrition of fungi has been reviewed recently by Foster (**1949a**). The fungus which has been most used in such studies, particularly of trace element requirements, in recent years, *Aspergillus niger* (see the many papers of Steinberg), produces large quantities of organic acids, a characteristic which is undesirable for reasons which will be discussed later. The Saprolegniaceae are suited to studies of inorganic requirements because they do not normally produce large quantities of organic acids, and grow in simple media.

The growth of the Saprolegniaceae in synthetic media has been studied by Volkonsky (**1932a** and **b**, **1933a** and **b**, **1934**), Saksena and Bhargava (**1941**), Bhargava (**1945a**, **b**, and **c**, **1946**), and Whiffen (**1945**). Volkonsky was primarily interested in sulfur, carbon, and nitrogen requirements; Bhargava and Whiffen reported experiments on phosphorus requirements and the absence of growth factor requirements as well. No systematic study of other elements in media used for the cultivation of these fungi has been made. The synthetic media used by previous investigators (TABLE 1) did not, in my hands, support as heavy growth of various members of the Saprolegniaceae as did a routine broth consisting of soluble

TABLE 1

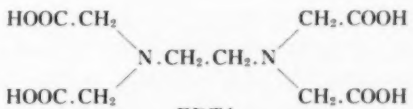
SYNTHETIC MEDIA IN WHICH SAPROLEGNIACEAE HAVE BEEN GROWN

Inorganic salts: K_2HPO_4 0.1 g./l. $MgCl_2$ 0.1 g./l. Fe_2Cl_6 0.01 mg./l.	KH_2PO_4 0.5 g./l. $MgCl_2.6H_2O$ 0.5 g./l. Na_2S 0.17 g./l. NH_4NO_3 2.0 g./l.	K_2HPO_4 } 0.005 M KH_2PO_4 } (pH 6.0) $MgSO_4.H_2O$ 0.002 M $FeSO_4$ 0.00002 M $ZnSO_4.7H_2O$ 0.00001 M $CaCl_2$ 0.001 M
Organic nutrients: cysteine HCl 0.05 g./l. alanine 1.0 g./l. glucose 1.0 g./l. NaOh to pH 7.0	glucose 5.0 g./l. pH 7.0	cystine 0.001 M glutamic acid 0.05% glucose 0.5% pH 6.0
Organisms grown: *Achlya conspicua* *A. oblongata* *A. polyandra* *A. prolifera* *Aphanomyces* sp. *Dictyuchus monosporus* *Isoachlya monilifera* *Saprolegnia parasitica* *Saprolegnia* sp.	*Achlya* sp. *Brevilegnia gracilis* [1] *Isoachlya anisospora* var. *indica* *Saprolegnia delica* *S. monoica*	*Achlya flagellata* *Aphanomyces stellatus* *Dictyuchus monosporus* *Saprolegnia ferax* *Thraustotheca clavata*
Volkonsky (**1933a**)	Bhargava (**1945b**)	Whiffen (**1945**)

[1] S as K_2SO_4, 0.5 g./l.

starch (0.5%), glucose (0.5%), and yeast extract (0.1%). An inorganic basal medium was therefore designed which supplied recognized essential elements as known components rather than as contaminants in other compounds added to the nutrient solution; it supported heavy and rapid growth over a wide range of pH on the addition of appropriate organic substrates.

The medium developed was based upon the use of ethylenediamine tetracetic acid (EDTA) as metal carrier. EDTA is a chelating agent



EDTA

forming soluble complexes with metallic ions (Schwarzenbach and Ackerman, **1948**). Complex formation presents precipitation of these ions at pH values at which precipitates would other-

wise occur. A standard text on quantitative analysis (Willard and Diehl, **1943**) gives the following as the pH values at which some metallic ions precipitate as hydroxides or basic salts: ferric ion, 2–3; zinc ion, 5.2; cupric ion, 5.4; ferrous ion, 5.5. If such precipitates do not form immediately in the absence of chelating agents, they may appear after autoclaving, withdrawing other essential metals as coprecipitants or by adsorption. This is the basis of Steinberg's (**1919, 1935**) technique for removing trace elements from nutrient solutions by adsorption on $CaCO_3$. The success of Steinberg in obtaining partial deficiencies of trace elements by this method should serve as warning against the use of media in which precipitates occur.

The difficulties encountered with iron were first recognized by Uspenski (**1927**), who introduced, on an empirical basis, the use of Fe-citrate to prevent precipitation of ferric hydroxide (**1925**). Fries (**1945**) showed that other metals, particularly calcium, behaved similarly as the pH was raised. Since the utilization and the toxicity of many substances are determined by the pH of the medium, the availability of the full physiological range of pH, made possible by the use of chelating organic acids (Fries, **1945**), is of obvious importance in nutritional studies. On the other hand, the chelating ability of citrate, tartrate, glycerol, etc., produced in fungal fermentations in varying quantities (as citric acid by *Aspergillus niger:* see Foster, **1949a**), must complicate research into the requirements of these fungi for inorganic ions participating in complex formation.

The replacement of citrate and other previously used chelating agents by EDTA, introduced by Schatz and Hutner (**1949**), has the advantages that EDTA is biologically inert (Hutner, Provasoli, Schatz, and Haskins, **1950**) and that it binds metallic ions more intensely. Tight chelation allows the supply of a larger reservoir of metallic ions in the medium, gradually becoming available by mass action during the growth of the organism. Tight chelation also provides a lower effective concentration of complex-forming contaminants in chemicals, water, glassware, or inocula. EDTA acts as a metal buffer, particularly useful at the higher pH values at which metallic ions precipitate, since intensity of binding increases with pH.

The inorganic basal medium developed for Saprolegniaceae had the following composition:

EDTA	0.05%
K_2HPO_4 | KH_2PO_4	0.03%
$MgSO_4.7H_2O$	0.1%
Ca (as Cl)	1.0 mg.%
Mn (as Cl)	4.0 mg.%
Zn (as Cl)	4.0 mg.%
Fe (as anhydrous $FeCl_3$)	0.1 mg.%
Cu (as $CuCl_2.2H_2O$)	0.2 mg.%
Mo (as $Na_2MoO_4.2H_2O$)	2.0 mg.%
B (as H_3BO_3)	2.0 mg.%
Co (as Cl)	0.1 mg.%

in distilled water. This medium must be at least partially neutralized during preparation to dissolve the free acid EDTA.

EDTA was obtained as "purified" free acid from the Bersworth Chemical Co., Framingham, Mass. The preparations of potassium phosphate (K_2HPO_4 and KH_2PO_4 in the same proportions as an equilibrium mixture at pH 7.0) and magnesium sulfate have been described by Hutner *et al.* (**1950**). Manganese metal, zinc metal, and cobalt chloride (solution B) were obtained as Johnson-Matthey "specpure" preparations. The metals were converted to chlorides by solution in redistilled HCl. Calcium chloride was prepared by weighing reprecipitated carbonate and dissolving with redistilled HCl, to avoid the considerable error which may result from attempting to weigh the chloride. Merck ferric chloride, resublimed, stored in a desiccator, was used. Copper, molybdenum, and boron, which could be omitted without affecting growth, were supplied as C.P. or reagent grade chemicals.

Nitrogen was supplied as NH_4NO_3 (for preparation see Hutner *et al.*, **1950**) at the level of 0.01%. Since the medium was brought to pH 8.0 or 8.5 with NH_3 vapor in a desiccator, in order to take advantage of the greater stability of EDTA-metal complexes at a more alkaline pH, more nitrogen was available than that added as ammonium nitrate. In experiments not concerned with inorganic nutrition, KOH pellets were used to raise the pH; nitrogen was not a limiting factor when either ammonium chloride or nitrate was supplied at 0.01%.

Methionine (DL-methionine, Winthrop Chemical Co.), since it is both stable and non-toxic, was supplied at the level of 2.5 mg.% as sulfur source. The Saprolegniaceae, with the exception of *Brevilegnia gracilis* (Bhargava, **1945a**), have been consistently reported as unable to reduce sulfate, though inorganic sulfur in the form of sulfide can be utilized. The undesirability of using sulfide, a metal precipitant, in a routine basal medium is indicated by the results of Volkonsky (**1932b**) and Bhargava (**1945a**). The inability of nine species of Saprolegniaceae (*Achlya bisexualis*, female, JRR 355; *A. bisexualis* ?, male, JRR E 247; *A. colorata*, LS; *A. flagellata*, AZ; *A. Klebsiana*, LS; *A. racemosa*, G–S; *Isoachlya intermedia*, IR–2; *Protoachlya paradoxa* LS; *Saprolegnia delica*, LS; and *Thraustotheca primoachlya*, AZ), obtained from Dr. J. R. Raper, Dr. L. Shanor, Dr. A. Ziegler, and Dr. E. K. Goldie-Smith (or isolated by the author), to reduce sulfate was confirmed. 5.0 mg.% of DL-methionine, added to a medium consisting of mineral base in which magnesium sulfate was replaced by magnesium chloride, 0.5% glucose, and 0.2% Na H glutamate, supported excellent growth of these species while Na_2SO_4, 0.01%, gave only the slight growth of the control to which no sulfur was added beyond the reduced sulfur compounds present in laboratory air. *Achlya Klebsiana*, LS, under conditions such that no growth occurred in eight days in the absence of added sulfur, did not grow with Na_2SO_4 in concentrations ranging from 0.1 to 100 mg.%, though duplicate flasks with 5.0 mg.% of DL-methionine grew well at all sulfate concentrations. The level of the methionine requirement of *A. Klebsiana* is indicated by table 2.

The basal medium given above was devised by determining the level of each component yielding most rapid and heavy growth and subsequently redetermining the requirement for each other component in the presence of slight excesses of those previously studied. This procedure of successive approximations was followed through two series of experiments: a preliminary series in which glucose (1.0% added aseptically after autoclaving) and Na H glutamate (0.1%) were used, and a series in which ethanol (0.5%, glass distilled, diluted and added aseptically) and Na_2 succinate·$6H_2O$ (0.25% Merck synthetic) replaced glucose and glutamate. Etha-

nol supported heavier and more rapid growth than such other easily-distillable substrates as acetate, glycerol, or butanol. Succinate was chosen of the acids of the Krebs cycle because it was available as a synthetic product of high purity; the role of Krebs cycle acids in the nutrition of Saprolegniaceae will be discussed in a later paper.

The organism used in these experiments was *Achlya Klebsiana* Pieters (**1915**). The stock culture was derived from a single hyphal tip of an isolate identified and sent by Dr. L. Shanor. This fungus was chosen because preliminary experiments indicated that it was unusual among the Saprolegniaceae in growing well at 35° C., and in the ease with which abundant sporulation could be induced.

TABLE 2

THE METHIONINE REQUIREMENT OF *Achlya Klebsiana in* MINERAL BASE (0.05% $MgCl_2.6H_2O$ SUBSTITUTED FOR $MgSO_4.7H_2O$) WITH 0.01% NH_4NO_3, 0.5% ETHANOL, AND 0.25% Na_2 SUCCINATE.$6H_2O$ AT pH 8.0

Growth was estimated five days after the beginning of visible growth. The notation ++++ indicates about 25–30 mg. dry weight.

Addition		Growth
none		0
DL-methionine	0.01 mg.%	±?
DL-methionine	0.05 mg.%	±
DL-methionine	0.2 mg.%	++⁺
DL-methionine	1.0 mg.%	++++
DL-methionine	2.5 mg.%	++++
DL-methionine	5.0 mg.%	++++

The cystospores and zoospores used as inoculum were produced when mycelial mats, washed in sterile distilled water, were placed into charcoal water (distilled water boiled with Merck activated charcoal which was subsequently removed by filtration) for twelve to twenty-four hours. These spores remained viable for at least two months at 5° C. when stored in the refrigerator after aseptic transfer to sterile screw-capped tubes.

One drop of spore suspension was added to 10 ml. of medium, distributed in 25-ml. Pyrex Erlenmeyer flasks (topped with inverted Pyrex beakers) placed in Pyrex baking dishes covered with another similar dish sealed to the first with transparent adhesive

cellulose tape. Flasks and beakers were prepared by boiling with a detergent for about half an hour, then rinsing with tap water several times for preliminary experiments or with distilled water in more critical experiments.

Rate and amount of growth were estimated by visual inspection, with occasional weighing of oven-dried mycelial mats to minimize subjective error. In the protocols which follow +*, ++ equals about 6 mg. dry weight, ++++ equals about 25–30 mg. dry weight. Experiments were conducted at 30 to 35° C. or in an incubator held at 30° C., when room temperature fell below this level. Growth was heavy and rapid at initial pH ranging from

TABLE 3

THE CALCIUM REQUIREMENT OF *Achlya Klebsiana* GROWN IN MINERAL BASE[1] PLUS METHIONINE WITH CALCIUM OMITTED, pH 8.5–8.6

Addition	Glucose 1.0% Na H glutamate 0.1%		Ethanol 0.5% Na_2 succinate.$6H_2O$ 0.25%
	2 and 5		5 Days after inoculation
none	0	0	0
Ca (as Cl) 0.04 mg.%	0	0	0
Ca (as Cl) 0.2 mg.%	0[2]	0	0
Ca (as Cl) 0.4 mg.%	—	—	0
Ca (as Cl) 0.5 mg.%	±	++++ (25.5 mg.)	—
Ca (as Cl) 0.8 mg.%	—	—	+
Ca (as Cl) 1.0 mg.%	++	++++ (28.1 mg.)	—
Ca (as Cl) 1.5 mg.%	—	—	++++
Ca (as Cl) 2.0 mg.%	++	++++ (28.3 mg.)	—

[1] In the experiment employing glucose and glutamate the mineral base used contained less manganese (1.0 mg.%) and zinc (3.0 mg.%) than the final base.
[2] Slight (±) growth at pH 8.0.

4.5 to 8.6, with no definite optimum, when appropriate organic constituents were added to the mineral base. There was no growth when the initial pH was 4.0; the upper limit of pH was not below pH 8.6. The previously reported optimum range of pH 4.2–5.8 (Whiffen, **1945**), which did not concord with the pH of habitats in which members of this family have frequently been found (see Lund, **1934**; Wolf and Wolf, **1941**; van Beverwijk, **1948**), is considered to be an artifact of the composition of the mineral solution employed.

The similarity of results in the two series of experiments, employing different substrates, demonstrates the metal-buffering ability of EDTA. The calcium requirement, however, changed markedly when substrates of biological origin (glucose and glutamate) were replaced by synthetic succinate and ethanol of considerably greater purity. More than twice as much calcium was required in ethanol-succinate media as in the presence of Na H glutamate (TABLE 3) which was appreciably contaminated with calcium, as shown by treatment with oxalate.

Although calcium has not been generally considered necessary for the growth of fungi, reports of stimulatory effects (Davis *et al.*, **1928**; Mann, **1932**; Lindeberg, **1944**; Fries, **1945**) were succeeded

TABLE 4

THE MANGANESE REQUIREMENT OF *Achlya Klebsiana* GROWN IN MINERAL BASE PLUS METHIONINE WITH MANGANESE OMITTED, pH 8.0

Addition	Glucose 1.0% Glutamic acid 0.08%		Ethanol 0.5% Na_2 succinate.$6H_2O$ 0.25%	
	1 and 7 days after inoculation		1 and 7 days after inoculation	
none	0	0	0	0
Mn (as Cl) 0.01 mg.%	0	0	0	+
Mn (as Cl) 0.04 mg.%	0	0	0	++
Mn (as Cl) 0.2 mg.%	0	+	0	+++
Mn (as Cl) 0.5 mg.%	0	±	0	+++
Mn (as Cl) 1.0 mg.%	0	+++	0	+++
Mn (as Cl) 2.0 mg.%	0	+++	±?	++++
Mn (as Cl) 4.0 mg.%	+	++++	±	++++
Mn (as Cl) 6.0 mg.%	—	—	±	++++

by the report of Steinberg (**1948**) that *Rhizoctonia solani* gave yields only 14.3% of normal when calcium (in trace amounts) was not supplied and that calcium deficiency had similar, though less marked, effects on four other fungi including the aquatic phycomycete, *Pythium irregulare*. Complete absence of growth, resulting from trace element deficiency, has been reported only on rare occasions (see Foster, **1949a**). The protocols of experiments concerned with calcium (TABLE 3) and manganese (TABLE 4) requirements of *Achlya Klebsiana* are presented here to emphasize the value of a strong solubilizing chelating agent in the development

of reproducible inorganic basal media. Experiments dealing with magnesium and zinc requirements yielded similar results: no growth in the absence of, or in the presence of insufficient amounts of, these ions. The concentrations given in the final medium are slightly in excess of the minimum amount required for most rapid and heavy growth of mycelial mats: a dry weight of about 30 mg. within five days after the beginning of visible growth. The growth obtained was entirely comparable with that in routine broth, though the lag phase was longer. Growth was barely visible the day after inoculation when CP chemicals were used, but a tube of broth inoculated as a check on the viability of the inoculum contained hyphae, approximately a centimeter long, growing up towards the surface. When the nutrient solution was made as indicated for experiments on inorganic nutrient requirements the lag phase was usually one or two days longer than in broth. The mineral base-methionine-ethanol-succinate-ammonium medium supported heavy growth of ten other species of Saprolegniaceae: *Achlya bisexualis*, JRR 355, *A. bisexualis* ? JRR E 247, *A. colorata* LS, *A. flagellata* AZ, *A. racemosa* G–S, *Brevilegnia unisperma* LS, *Dictyuchus monosporus* DS–1, *Isoachlya intermedia* IR–2, *Protoachlya paradoxa* LS, *Saprolegnia delica* LS, and *Thraustotheca primoachlya* AZ) as well as of *A. Klebsiana* LS.

Since no requirement for copper, molybdenum, boron, or cobalt could be demonstrated under the conditions of these experiments, the mineral base might be simplified by the omission of these elements in experiments not designed to deal with inorganic nutrition, when CP chemicals, ordinary distilled water, and Pyrex glassware are used. The requirement for iron is adequately satisfied by 0.1 mg.% ferric ion; inocula not containing charcoal water did not grow in the absence of added iron in a later experiment, but reproducible growth curves varying with iron supply were not obtained in the present series of experiments. Ferric iron chelates only weakly.

It is probably unnecessary to point out that the levels of requirements demonstrated in these experiments are not absolute, even if one neglects, as is usual, the changes in the medium caused by the growth of the organism. While approximations of absolute re-

quirements could be made either by varying the chelating agent and extrapolating to zero chelator (see Hutner, **1948**) or by calculation from a series of equations employing the relative dissociation constants of the entire complex of chelating agent and various metallic ions, a more serious problem is the incompleteness of the list of known essential elements. The lengthening of the lag phase as more refined chemicals were used could not be compensated for by raising the concentration of any chemical in the medium, though toxicity was not evident before the metal-buffering capacity of 0.05% EDTA was exceeded and the formation of precipitates coincided with the absence or inhibition of growth. The addition of 2.0 mg.% of zinc, or 2.0 mg.% of manganese, or 1.8 mg.% of copper, or 1.9 mg.% of iron had this effect, indicating the width of the margin of safety available when natural materials containing metallic contaminants are added to the basal medium.

The elements for which no present necessity could be demonstrated were retained in the medium in order to avoid the possibility of multiple deficiencies which might accompany a change in the preparation of water, chemicals, or culture containers. Evidence for the essentiality of copper, molybdenum, and gallium in fungal nutrition is presented by Steinberg (**1945**), who does not consider boron necessary. Gallium (Johnson-Matthey "specpure" solution B of chloride) in concentrations of 0.05, 0.2, or 1.0 mg.% did not affect the growth of *Achlya Klebsiana*. There is some evidence that thallium, vanadium, and a variety of other elements as well as gallium should be included in a complete mineral base: "One can say about almost any chemical element that appears not to be essential only that it is not required in greater quantity than is represented by the unavoidable impurities in the culture solution . . ." (Hoagland, **1948**).

The advantages of this inorganic basal medium for the growth of Saprolegniaceae are its reproducibility or relative freedom from effective change when changes are made in sources of salts or in substrates, its freedom from precipitates at relatively high pH, and the improvement in growth over that supported by mineral basal media previously used. The usefulness of the extended pH range for growth is illustrated by the effect of acetate on growth. Tox-

icity and non-utilization of acetate at pH 6.0 were reported by Whiffen (**1945**). At pH 4.8 0.01% Na acetate·$3H_2O$ gave the mycelium of *Achlya Klebsiana* (isolate AcS–12) the appearance of a poorly fixed histological specimen and nearly completely abolished endogenous respiration (oxygen uptake) within four hours. At pH 7.0 sodium acetate, presumably present to a greater extent as the less readily penetrating acetate ion, was a fair source of carbon and energy for growth in concentrations up to 0.5%; higher concentrations were not tested.

At the conclusion of these experiments it was also observed that the mineral base plus DL-methionine would replace the chemically undefined decoction, charcoal water, in sporulation. Spore production was poor in distilled water, in distilled water with the addition of calcium salts, or diluted Hoagland's or Pfeffer's solutions, and not quite as good with 0.02% Na citrate·$5\frac{1}{2}H_2O$ as with charcoal water. The use of charcoal water for producing zoospores was based upon the idea that distilled water was made non-toxic by the adsorption of injurious metals upon the charcoal. It has been recognized that charcoal also contributes other substances to the water, but exception has not been taken to the classic remarks of Klebs (**1899**) that zoospore production occurred whenever a healthy mycelium was transferred to pure water and was suppressed by extremely low concentrations of nutrients or moderate concentrations (0.1% or less) of inorganic salts. Since charcoal water is hardly pure water, it is interesting to note that a medium which allowed growth, but contained suboptimal amounts of trace elements, notably zinc and manganese, was inadequate for zoospore production, though sporangial initials were delimited. The inorganic medium given here allowed zoospore production by all of the members of the Saprolegniaceae for which it supported growth in the presence of added substrate; even the centric *Achlyas*, which are characterized in part by infrequency of zoospore production, respond to this treatment. The contribution of charcoal water in its other mycological roles: in chytrid cultures, in the germination of myxomycete spores (Elliott, **1949**) or of oospores of the Saprolegniaceae (Ziegler, **1948**), may be quite different. The production of zoospores, however, is suppressed by metal de-

ficiencies as well as by toxicities, as is sporogenesis in, for example, *Bacillus cereus* (Foster, **1949b**), or among terrestrial fungi (Foster, **1949a**).

SUMMARY

An inorganic mineral base was designed, employing ethylenediamine tetracetic acid as metal carrier, which supported heavy growth (on the addition of appropriate organic substrates and ammonium) over a wide range of pH, and sporulation of 11 species of Saprolegniaceae. No growth of *Achlya Klebsiana* occurred if magnesium, calcium, zinc, manganese, iron, or a sulfur source (DL-methionine) were omitted. The species investigated were unable to reduce sulfate. Although unknown limiting factors, probably inorganic nutrients, increased the lag phase of growth when the purest available chemicals were used, copper, molybdenum, cobalt, boron, and gallium did not appear essential or stimulatory under the conditions of these experiments.

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CROSSES INVOLVING BIOCHEMICALLY DEFICIENT MUTANTS OF ALLOMYCES ARBUSCULA

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The production of mutant strains with biochemical deficiencies in the water mold *Allomyces arbuscula* would be of great value in clarifying the life cycle of this organism, and might aid in making certain crosses between different strains, if these strains carried different biochemical potentialities. The work reported here concerns the production of such biochemical mutations in a strain of *A. arbuscula* (Philippine Islands No. 1) obtained from Dr. R. Emerson, and the results of several crosses between these deficient strains.

Eight single zoospores were isolated from germinating resistant zoosporangia of this strain. The colonies arising from these single zoospores appeared identical in all respects and therefore one isolate (No. 3) was arbitrarily chosen, and progeny from this isolate were used throughout the study. The composition of the minimal and complete media used is given below.

MINIMAL AND COMPLETE MEDIA FOR *A. arbuscula* (PHIL. ISL. NO. 1)

Minimal		Complete	
Glucose	1 g.	Glucose	1 g.
Asparagine	0.1 g.	Asparagine	0.1 g.
$MgSO_4$	0.05 g.	$MgSO_4$	0.05 g.
M/150 KH_2PO_4	50 ml.	M/150 KH_2PO_4	50 ml.
M/150 Na_2HPO_4	50 ml.	M/150 Na_2HOP_4	50 ml.
dl-Methionine	2.5 mg.	Yeast extract (Difco)	0.4 g.
Thiamin	1 μg.		
pH before autoclaving—6.8		pH before autoclaving—6.8	

When solid media was desired two per cent water washed agar was added to the formula above. Growth was also obtained upon this medium when synthetic dl-β-asparagine replaced the l-asparagine ordinarily employed. The cultures were grown in 20 ml. of

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medium in 125 ml. Erlenmeyer flasks at 25° C. for seven days when the growth factor requirements were being determined.

Since the R. S. zoosporangia of this strain release most of their zoospores over a period of 5–6 hours, it was essential that irradiation be carried out before the zoospore nucleus had divided, because the presence of any mutant genes produced in one nucleus might be masked by the presence of their wild type alleles in the heterocaryotic germlings. For this reason R. S. zoosporangia, which had been dried for one month on sterile filter paper in tubes and then suspended in 10 ml. of sterile water at the time of the experiment, were allowed to discharge zoospores for 2 hours, after which the zoosporangia were centrifuged leaving the zoospores suspended in the supernatant fluid. The R. S. zoosporangia were resuspended and this process repeated twice. A control sample of zoospores was removed prior to each irradiation.

The R. S. zoosporangia, suspended in distilled water in a quartz test tube, were irradiated with ultra violet rays from a germicidal lamp with approximately 90 per cent radiation at 253.7 mμ for 7 minutes with the material 12 inches from the light source. This irradiation killed approximately 80 per cent of the zoospores. The three lots of irradiated spores were combined, diluted and plated out on solid minimal medium and allowed to grow at 25° C. for 48 hours. The colonies which developed were marked, and then the layer of minimal agar containing the colonies was covered with a layer of complete agar. After 48 hours the colonies which had appeared upon the addition of the complete medium were isolated. The control samples of unirradiated spores were handled in a similar way. From 9615 colonies which survived irradiation, 11 colonies were isolated which failed to grow in the minimal medium but would grow in the complete medium. All of these mutant isolates would also grow in the minimal medium upon the addition of the following amino acids: arginine, lysine, valine, threonine, phenylalanine, histidine, leucine, isoleucine, and tryptophan. Two of these biochemical mutants designated No. 7 and No. 23 were selected for further work.

Strain No. 23 produced more aerial mycelium than the wild type, but the rate of gamete release and conjugation was compara-

ble. R. S. zoospores and conjugated gametes, while germinating in minimal medium, required lysine to achieve colonial growth comparable to that upon complete medium. The optimum amount of l-lysine was 5 mg./100 ml. of minimal medium.

Strain No. 7 produced convex colonies on solid medium rather than the spreading type of growth exhibited by wild type colonies. The growth rate of the gametophytic thallus was similar to wild type and gamentangium formation and gamete release were normal, but the number of sporophytic colonies developing from conjugated gametes was less than that of the wild type. This strain would, however, cross readily with either wild type or No. 23. The failure to produce many sporophytic colonies from conjugated gametes may be due to the presence of a semi-lethal factor. This strain required the addition of arginine to minimal medium, although growth in minimal plus arginine was slower than in the complete medium. The optimum amount of dl-arginine was 5 mg./100 ml. of minimal medium.

Mutants No. 7 and No. 23 could be crossed by mixing gametes for an hour or two in sterile water and plating out in minimal medium. In this case only the recombination types grew, since the selfed-combinations were deficient for lysine or arginine. Crosses of wild type × No. 7 or × No. 23 were made by mixing female gametangia of wild type with male gametangia of the mutants, allowing the gametes to be released and plating them out in complete medium.

TABLE 1

SEGREGATIONS IN R. S. ZOOSPORANGIA FROM CROSSES OF WILD TYPE OF *A. arbuscula* × MUTANTS AND MUTANT × MUTANT

Crosses	Gametophyte colonies tested	Gametophyte colonies growing			
		Minimal medium	Minimal medium +arginine	Minimal medium +lysine	Minimal medium +lysine and arginine
Wild type × No. 23	131	72	—	59	—
Wild type × No. 7	97	62	35	—	—
No. 7 × No. 23	24 (from single R. S. zoosporangium)	12	6	4	2

Table 1 indicates that the cross, wild type × No. 23, segregated in a 1 : 1 ratio, as would be expected if the lysine requirement was due to mutation of a single gene. The cross, wild type × No. 7, only approximated a 1 : 1 segregation, but the ratio here might well be affected by the lethal factor previously mentioned. The cross of the mutant types No. 7 × No. 23 did not give a 1 : 1 segregation. Some possible explanations for this unexpected ratio are decreased viability of the mutant strains, possible reverse mutations in the progeny of the cross, or the clumping of the R. S. zoospores used in securing the progeny for the growth requirement tests. In the latter case mutant and wild type colonies growing in close proximity would appear only as wild type.

The evidence presented above indicates that meiosis must occur in the R. S. sporangium and that the resulting R. S. zoospores give rise to haploid colonies under the conditions studied. This confirms Emerson's earlier analysis of the life cycle of this organism. The question as to whether the sporophytic mycelium bearing the zoosporangia is diploid or merely heterocaryotic with two types of haploid nuclei remains to be answered. It has not been possible to demonstrate a requirement for lysine in any of the colonies arising from the germination of evanescent zoosporangia on the sporophytic plants of the cross wild type × No. 23. All these isolated colonies grew upon minimal medium. However, in crosses of wild type × No. 7, it has been possible to isolate, from the zoospores of a few evanescent zoosporangia, colonies requiring arginine. Examination of zoospores stained with the Giemsa technique showed that the zoospores in this cross were predominantly uninucleate, although occasional binucleate zoospores did occur. The presumption must be that the sporophytic thallus is generally diploid although abnormalities may exist.

The most difficult problem encountered in working with this strain of *A. arbuscula* (Philippine Islands No. 1) has been to obtain proper maturation of the R. S. zoosporangia and the release of the R. S. zoospores. In one of the early cultures examined 70 per cent of the R. S. zoosporangia released zoospores, but since that time only 1–20 per cent of the R. S. zoosporangia of the wild type and of No. 23 have released zoospores, and long periods are re-

quired for their maturation. Out of 32 attempts using large numbers of R. S. zoosporangia of various ages produced on sporophytic colonies derived from selfed-conjugants of No. 7, only 3 gametophyte colonies have ever grown.

It is a pleasure to acknowledge here the cooperation of Dr. Ralph Emerson of the University of California in providing the cultures upon which this work was based and for several valuable suggestions concerning the behavior of these mutant crosses.

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VARIOUS ZOÖPAGACEOUS FUNGI SUBSISTING ON PROTOZOANS AND EELWORMS

CHARLES DRECHSLER [1]

(WITH 6 FIGURES)

Several animal-destroying conidial phycomycetes are reported herein that came under observation in agar plate cultures following the addition of small quantities of decaying vegetable detritus. Two of the fungi, both endoparasitic in *Amoebae*, are being described as new members of the Zoöpagaceae, one of them being readily assigned to the genus *Cochlonema*, while the other differs sufficiently from related forms to merit recognition as the type of a separate genus. Some discussion is given to two other species which subsist endoparasitically on protozoans and embody features that in some degree extend the scope of morphological diversity in the family. The earlier account of my *Acaulopage lophospora* (**10**: 133–137) is amplified by a description of the sexual stage belonging to that fungus. Incomplete observations on a nematode parasite referable to the curious genus *Euryancale* are recorded.

A COCHLONEMA WITH ZYGOSPORANGIA HAVING DISTALLY LOBED COLUMNAR PROTUBERANCES

A maizemeal-agar plate culture which after being overgrown with mycelium of *Pythium ultimum* Trow had been further planted by adding a few pinches of decaying marsh grass (*Spartina* sp.) detritus taken up near Mayo, Maryland, on August 20, 1946, showed three weeks later some scattered *Amoebae* that contained spirally convolved thalli of a zoöpagaceous parasite (FIG. 1, *A–E*). The infected animals usually measured 45 to 70 μ in width when

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they were drawn into a rounded shape. Each was enveloped by a thin pellicle which for the most part appeared cast into broadly undulating folds. Each contained a single contractile vacuole (FIG. 1, *B–D: v*). In newly invaded specimens the finely granular protoplasm surrounded a prolate ellipsoidal nucleus (FIG. 1, *B, n*), often 18 to 24 μ long and 11 to 14 μ wide, that showed slightly dark elongated bodies arranged peripherally, close under the delimiting membrane. With respect to shape and internal organization, therefore, the nucleus here corresponded fairly well to that of *Amoeba terricola* Greeff—the rhizopod earlier found captured by my *Acaulopage marantica* (**7**: 143–149) and also parasitized both by my *Endocochlus gigas* (**5**: 368–371) and my *Cochlonema megaspirema* (**6**: 235–241). In infected animals where the fungus was found in a more advanced state of vegetative development the peripheral bodies often seemed shorter and more nearly round (FIG. 1, *C, n; D, n*), thereby in some degree approaching the shape and dimensions of the peripheral bodies in the nucleus of the animal attacked by my *Cochlonema euryblastum* (**9**: 283–289). Perhaps in such instances the peripheral bodies may have undergone pathological modifications, especially as the interior of the nucleus was noticeably abnormal in its darkened mottled appearance. Nevertheless, the identity of the animal with Greeff's species cannot be regarded as wholly certain.

The manner in which the fungus gains entrance into its protozoan host has never come directly under observation. However, since the thallus has never been found with an empty conidial envelope attached to it, infection most probably takes place as in *Endocochlus gigas*. At an early stage in its development the thallus is recognizable as a prolate ellipsoidal body (FIG. 1, *A, a*) about 10 μ long, either straight or slightly curved along its major axis. On elongating it soon shows rather pronounced curvature (FIG. 1, *A, b*). When it attains a length of 20 to 30 μ its growing tip often reaches a position near its proximal end, thereby forming a tight coil of a single turn (FIG. 1, *B, a; C, a*). Although some further elongation often takes place nearly in the plane of the first turn (FIG. 1, *C, b, c*), the second turn for the most part is applied somewhat laterally to the first (FIG. 1, *C, d*).

Usually when a coil of 2 or 2¼ turns has been formed the hypha bifurcates distally (FIG. 1, *A*, *c–e*; *C*, *d*). The two branches, after describing an additional quarter (FIG. 1, *A*, *c–e*) or half turn (FIG. 1, *D*, *a*), again bifurcate. A third (FIG. 1, *D*, *a*) and a fourth dichotomy (FIG. 1, *E*) often ensue, following increases in length not greatly exceeding 15 μ. In each dichotomy the width of the resulting branches is noticeably reduced. The narrowed branches of the second, third, and fourth orders show increasing curvature, thereby adding to the distinctive appearance of the massive hyphal clew (FIG. 1, *D*, *a*; *E*). Owing to general similarities in the convolvement and branching of its thalli, the fungus recalls more especially *Endocochlus gigas*, *Cochlonema eurybiastum*, and my *Cochlonema agamum* (**10**: 120–133).

Like many related forms the fungus begins asexual reproduction when the animal host becomes incapable of further locomotion, mainly from loss of protoplasm. While assimilation of the remaining host material continues the vegetative thalli put forth slender reproductive filaments. A small thallus usually gives off a single reproductive filament from a position near its proximal end and on the outer convex side of the coil (FIG. 2, *A*, *a*; *B*, *a*). Larger thalli give rise commonly to several reproductive filaments (FIG. 2, *C*, *a–d*) from positions mostly 5 to 10 μ apart, along the convex side of the first turn of the coil. Many reproductive filaments, after breaking through the host pellicle, push their way through the overlying or surrounding solid materials, and then elongate in the air. The submerged portions of such filaments show no outward modification, but in the aerial prolongations narrowed constrictions spaced at regular intervals set off a series of expanded portions (FIG. 2, *D*). As the reproductive filaments continue their growth the thallus supplying them becomes more and more vacuolate (FIG. 2, *A–C*). When the thalli have been emptied of all living contents, terminal growth in the filaments ceases and each aerial prolongation is converted into a chain of spores by the laying down of cross-walls in the narrowed isthmuses (FIG. 2, *E*, *a–d*). On slight disturbance the chains break up, leaving the substratum strewn with conidia (FIG. 2, *F*, *a–z*; *G*, *a–l*) ready to attack any suitable host animal that may come. In their somewhat

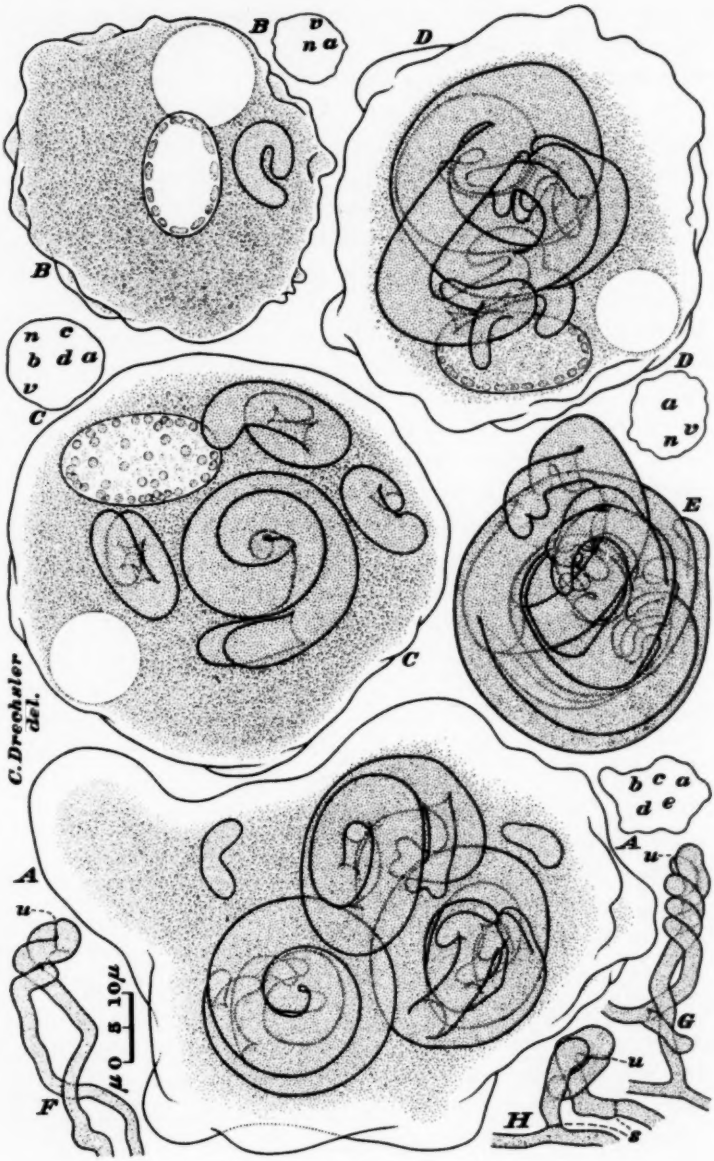
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FIG. 1.

cylindrical shape and slightly warty sculpturing the conidia resemble those of my *Cochlonema odontosperma* (**6**: 229–235) and of my *C. megalosomum* (**7**: 128–137). Their dimensions would seem a little larger than the corresponding dimensions of *C. odontosperma*, and a little smaller than those of *C. megalosomum*.

Sexual reproduction is initiated by the pairing of some reproductive hyphae soon after they have broken through the host pellicle. As far as could be determined from the meager material available, the paired hyphae regularly have their origin in separate thalli. Once they have made apical contact with each other, the two hyphae grow conjointly in length, winding about one another with either right-handed (FIG. 1, *F*) or left-handed rotation (FIG. 1, *G*). Often, again, the two hyphae show no spiral intertwining, but are interlocked in a more haphazard manner by means of irregular lobes or short lateral branches (FIG. 1, *H*; FIG. 2, *H*). Through deposition of a wall (FIG. 1, *H*, *s*; FIG. 2, *H*, *s*) in each of the hyphae a pair of gametangia are delimited which become united when the membranes at the contiguous tips (FIG. 1, *F*, *u*; *G*, *u*) are dissolved (FIG. 1, *H*, *u*; FIG. 2, *H*, *u*). A globose excrescence thereupon buds out laterally from one of the gametangia in a position close to the union. This excrescence gradually expands to a diameter of 12 to 15 μ. In the later stages of its growth it puts forth many columnar protuberances that terminate individually in three or four well marked lobes (FIG. 2, *H*, *c*). The

FIG. 1. *Cochlonema calosperma*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Specimen of *Amoeba* sp. (possibly *A. terricola*) containing two very young thalli, *a* and *b*, of the fungus, as well as three larger thalli, *c–e*. *B*, Specimen of *Amoeba* sp. (possibly *A. terricola*) containing a rather small thallus, *a*, of the parasite; *n*, host nucleus; *v*, contractile vacuole of animal host. *C*, Specimen of *Amoeba* sp. (possibly *A. terricola*) containing three coiled unbranched thalli, *a–c*, and a large coiled thallus with one bifurcation, *d*; *n*, nucleus of animal host; *v*, contractile vacuole of animal host. *D*, Specimen of *Amoeba* sp. (possibly *A. terricola*) containing a large thallus, *a*, bifurcating successively three times; *n*, nucleus of animal host; *v*, contractile vacuole of host. *E*, Large thallus with four successive bifurcations. *F*, Pair of sexual branches intertwined with right-handed rotation; *u*, apical contact between the branches. *G*, Pair of sexual branches intertwined with left-handed rotation; *u*, apical contact between the branches. *H*, Pair of sexual branches not spirally entwined; *s*, cross-walls delimiting the two gametangia basally; *u*, place of union between gametangia.

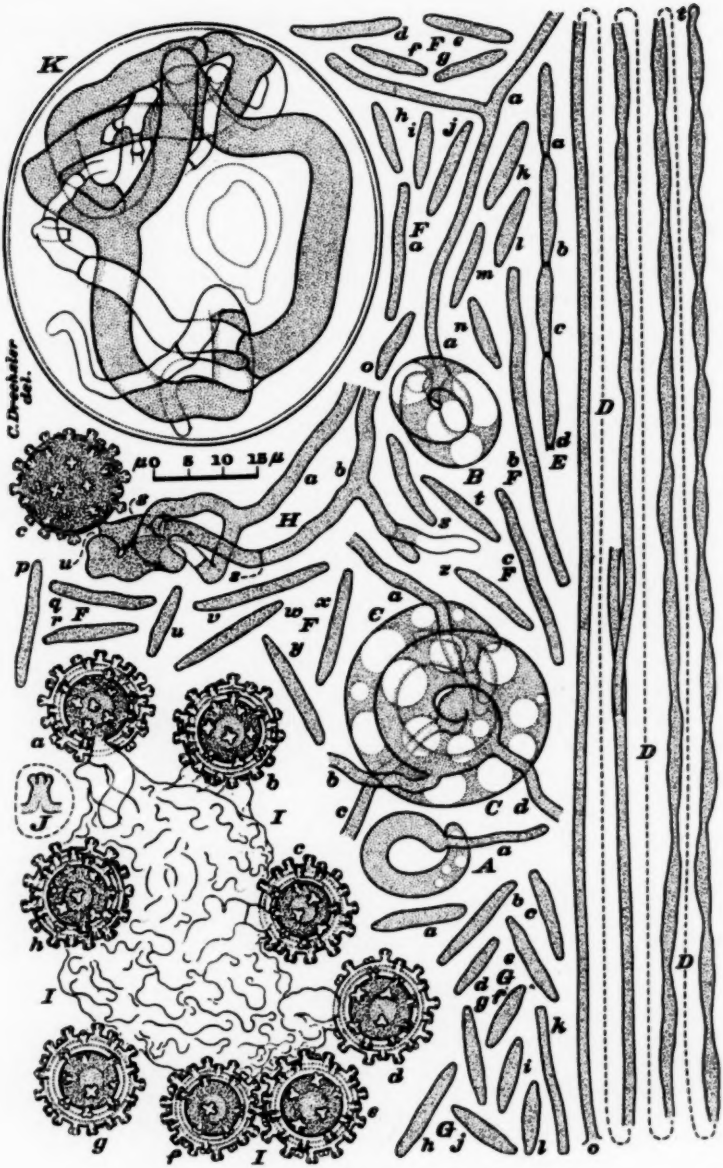

FIG. 2.

handsomely ornamented globose body now undergoes the usual internal changes entailed in the ripening of a zygospore. At maturity it contains a spherical protoplast often 8 to 9 μ in diameter (FIG. 2, *I, a–h*). This protoplast seems composed of a homogeneous central mass and a parietal layer of granular texture. Further details in structure of protoplast and wall are alike obscured through the presence of the many protuberances, which apparently consist of solid wall material (FIG. 2, *J*). It remains uncertain whether a single thick envelope of plural layers lies between the protoplast and the protuberances, or whether a zygospore wall proper is loosely surrounded by a separate zygosporangial envelope.

A term having reference to the handsome and very distinctive ornamentation of the sexual reproductive body is deemed appropriate as specific name for the fungus.

Cochlonema calosperma sp. nov.

Hyphae assumentes incoloratae, primo continuae, in modum cornus dilatatae, 3–11 μ crassae, usque 150 μ longae, in spiram cochleatim semel vel bis vel ter volutae, interdum (plerumque quandocumque tantummodo semel vel bis volutae) simplices interdum (plerumque quandocumque bis vel ter volutae) semel usque quater repetite dichotomae, prope originem saepe 1–4 quandoque plures hyphas genitabiles emittentes; hyphis genitabilibus 1.2–2 μ crassis, animali debilitato vel moribundo pelliculam ejus perforantibus, denique in aërem catenulas conidiorum ascendentes proferentibus aut in materia subjacenti vel ambienti ramos zygosporiferos gignentibus; conidiis incoloratis,

FIG. 2. All parts except *J* drawn to a uniform magnification with the aid of a camera lucida; × 1000 in all parts except *J*. *A–I, Cochlonema calosperma: A, B,* Small unbranched thalli from each of which a reproductive hypha, *a,* is being extended. *C,* Large coiled thallus from which four reproductive hyphae, *a–d,* are being extended. *D,* Reproductive hypha having its origin, *o,* in a thallus; its proximal sterile portion showing little variation in width but its distal portion showing successive constrictions at which conidia will later be delimited; *t,* growing tip; for lack of space the filament is shown in four parts whose proper connection is indicated by broken lines. *E,* Distal portion of conidial chain, showing four separate conidia, *a–d. F* (*a–z*), *G* (*a–l*), Random assortment of detached conidia. *H,* Immature unit of sexual apparatus, showing: *a, b,* the two sexual branches; *c,* the nearly fully grown zygosporangium with its distally lobed protuberances; *s,* cross-walls delimiting the gametangia basally; *u,* place of union between gametangia. *I,* Empty *Amoeba* pellicle around which are grouped eight mature zygospores, *a–h*. *J,* Protuberance of zygosporangium, showing lobed distal end; about × 2500. *K,* Testa of *Arcella discoides* containing a mycelium of a zoöpagaceous fungus.

cylindraceis vel elongato-ellipsoideis, plerumque leviter verrucosis, rectis vel leviter curvis, 10–47 μ (saepius 10–27) longis, 1.5–2.7 μ latis; ambobus ramis zygosporiferis vulgo ex aliis hyphis assumentibus oriundis; gametangiis saepe circa 25 μ longis, sursum 2–4 μ rarius usque 5 μ latis, ambobus quandoque sed non semper inter se spiraliter in modum caulis Phaseoli vulgaris vel in modum caulis Humuli lupuli circumvolutis, apice conjugentibus, denique prope junctionem zygosporangium emittentibus; zygosporangio globoso, 25–45 prominentiis speciose ornato, sine prominentiis vulgo 12–15 μ crasso, in maturitate flavidulo, cellulam viventem 8–9 μ crassam circumdante; prominentiis columnaribus, 1.5–2 μ longis, apice in 3–4 ramulos rotundos abeuntibus.

Amoebam saepe 45–70 μ latam (forsitan Amoebam terricolam) enecans habitat in foliis caulibusque Spartinae putrescentibus prope Mayo, Maryland.

Assimilative hyphae colorless, originally continuous, widening out from the narrowly rounded proximal end in the manner of a horn, usually 3 to 11 μ in width, sometimes as much as 150 μ long, coiled in a snail-like spiral of 1 to 3 successive turns, when composed of only 1 or 2 turns often simple, but when comprising 2 to 3 successive turns more usually bifurcate or two, three, or four times successively dichotomous, putting forth from the convex profile near the proximal end 1 to 4 (sometimes more) reproductive filaments 1.2 to 2 μ wide, which, after disablement of the host animal, push through its pellicle either to extend into the air somewhat moniliform prolongations that later are converted into conidial chains or to produce zygophoric branches in the underlying or surrounding material. Conidia colorless, cylindrical or elongated ellipsoidal, usually with a slightly warty lateral outline, straight or slightly curved, 10 to 47 (mostly 10 to 27 μ) long, 1.5 to 2.7 μ wide. Zygophoric branches in a pair commonly originating from separate assimilative hyphae, each by deposition of a cross-wall supplying a terminal gametangium; gametangia about 25 μ long, distally 2 to 4 μ (rarely up to 5 μ) wide, the two of a pair sometimes spirally intertwined with right-handed or left-handed rotation and sometimes more haphazardly interlocked by means of short branches or protuberances, after fusing apically burgeoning forth a zygosporangium near the union; zygosporangium subspherical, handsomely ornamented externally with 25 to 45 columnar protuberances, exclusive of the protuberances measuring usually 12 to 15 μ in diameter, at maturity containing a subspherical protoplast 8 to 9 μ in diameter; protuberances mostly 1.5 to 2 μ long, terminating in 3 or 4 subglobose lobes.

Parasitic on an *Amoeba* often 45 to 75 μ wide (probably *Amoeba terricola*) it occurs in decaying leaves and culms of *Spartina* sp., near Mayo, Maryland.

AN AMOEBA PARASITE WITH A DESMID-LIKE THALLUS

A maizemeal-agar plate culture which after being overgrown with mycelium of *Pythium ultimum* Trow had been further planted by adding a small quantity of decaying willow (*Salix* sp.) leaf detritus gathered in a moist thicket near College Park, Maryland, on February 5, 1950, showed on examination 14 days later, numerous small specimens of *Amoeba* undergoing destruction by an endoparasitic fungus that in scattered positions pushed up into the air small groups of delicate spore chains composed of small conidia. The animals attacked measured 20 to 25 μ in width when drawn into a rounded shape (FIG. 3, *A–E*). They were observed only on the surface of the agar, never being seen moving about under the surface. Their finely granular protoplasm was surrounded by a delicate pellicle with a gently undulating or minutely rippled contour. Apart from their single contractile vacuole (FIG. 3, *A, v; C–E: v*), they showed internally a prolate ellipsoidal nucleus (FIG. 3, *A–E: n*), usually 4 to 5.3 μ long and 3.3 to 4.3 μ wide, within which a slightly darker, globose endosome, 1.8 to 2.5 μ in diameter, was distinguishable. Evidently they all belonged to a single species of *Amoeba* generally resembling *A. sphaeronucleolus* Greeff and *A. verrucosa* Ehrenberg in nuclear make-up, but having dimensions much smaller than either of these familiar rhizopods. Many individuals, whether healthy (FIG. 3, *A*) or infected (FIG. 3, *B–F*), contained a variable number of colorless subspherical bodies, mostly 2.3 to 3.5 μ in diameter, which from their close resemblance to other such bodies strewn about abundantly everywhere on the agar plate seemed best interpretable as ingested fungus spores.

At the earliest stage of vegetative development in which the parasite can be recognized with certainty, its thallus is often bulb-shaped and consists of a globose part measuring about 4 to 5 μ across, together with a narrower part approximately 1.5 μ in width (FIG. 3, *B, a*). In all likelihood the narrower part here represents the infective body formed at the tip of a germ tube intruded into the protoplasm from an externally adhering conidium. Apparently it soon expands as nourishment is assimilated, for after some increase in size the thallus often has an elongated ellipsoidal outline (FIG. 3, *B, b; C, a, b*) and then offers a little resemblance

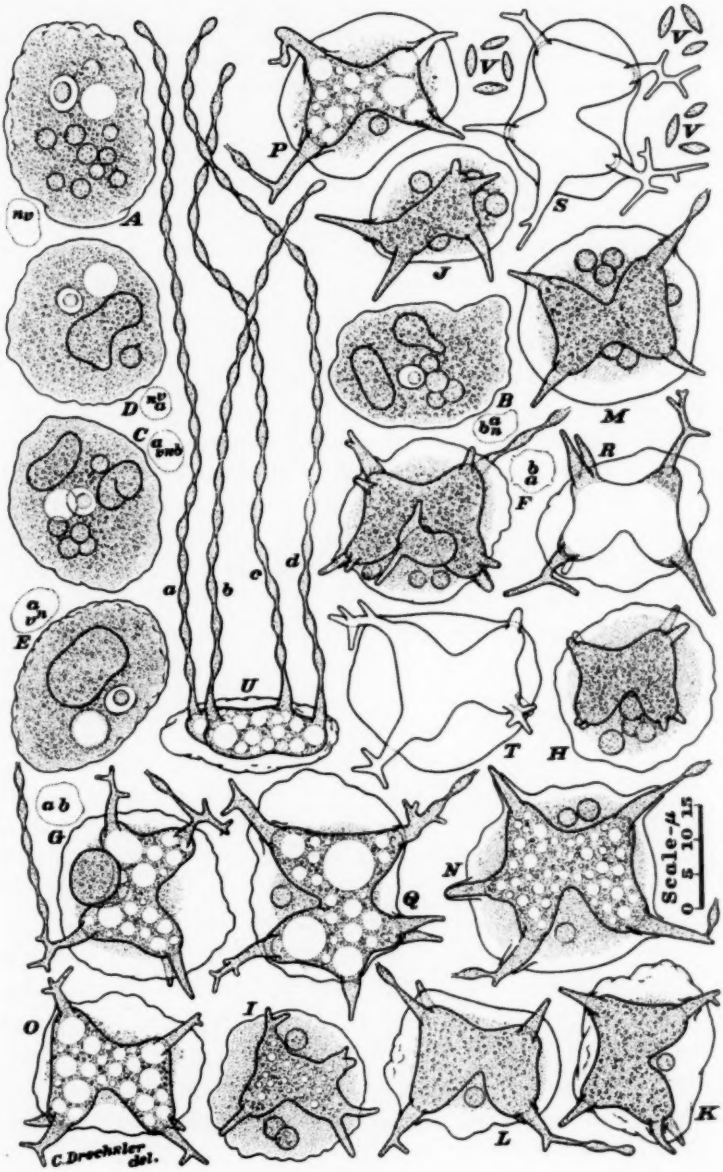
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FIG. 3.

to young thalli of *Cochlonema* or *Endocochlus*. Further growth, however, takes place not through elongation at the distal end as in the two helicoid genera, but through horizontal extension at the margin, so that the thallus acquires a flattened pillowy shape (FIG. 3, *D, a; E, a*). Where two thalli are present in an animal (FIG. 3, *F, a; G, a*) the younger smaller one may find its source of nourishment exhausted early, and may therefore need to become modified for sporulation while still in a juvenile form. More usually only one infection occurs, and the single thallus alters its pillowy shape by expanding unequally in such wise that a major constriction is left midway of its length, and a minor indentation is left at either end (FIG. 3, *F, b; G, b*). From the several arms of the desmid-like body (FIG. 3, *H–T*) tapering hyphal outgrowths are extended that break through the enveloping pellicle to elongate into the air as erect or ascending filaments made up of many minutely verrucose swollen portions connected by narrowed isthmuses (FIG. 3, *U, a–d*). When the swollen portions are separated from one another by the laying down of delimiting walls in the constrictions, each aerial filament is converted into a chain of conidia. The several chains break up on slight disturbance, scattering the individual spores (FIG. 3, *V*) about on the substratum in positions favorable for lodgment on new host animals.

Although microscopical scrutiny with a dry objective most usually shows four conidial chains arising close together from an in-

FIG. 3. *Aplectosoma microsporum*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Normal specimen of host *Amoeba; n*, nucleus; *v*, contractile vacuole. *B*, *Amoeba* host containing two small thalli, *a* and *b; n*, nucleus of animal. *C*, *Amoeba* host containing two small thalli, *a* and *b; n*, host nucleus; *v*, contractile vacuole of animal host. *D, E*, *Amoebae*, each occupied by a single thallus of larger size, *a; n*, host nucleus; *v*, contractile vacuole of host. *F, G*, *Amoeba* hosts, each containing a small thallus, *a*, and a large thallus, *b*, from which reproductive hyphae are being extended. *H–R*, *Amoeba* hosts, each containing a well-developed thallus; the several thalli showing increasing vacuolization as contents are utilized in development of reproductive hyphae and conidia. *S, T*, Pellicles of host *Amoebae*, each empty of protoplasm and occupied only by the empty membranous envelope of a thallus whose contents were spent in producing conidia. (In *F–T* the reproductive outgrowths and sterigmata are shown flattened out considerably, as seen in specimens under a cover-glass when viewed from above.) *U*, Lateral view of host pellicle and of thallus with four ascending reproductive hyphae, *a–d*. *V*, Detached conidia.

fected animal, groups of five chains occur with some frequency. Such groups generally come from thalli of more than ordinary size (FIG. 3, *N, Q, S*) in which the sequence of development was modified to form five rather than four divergent lobes, and accordingly also five primary hyphal outgrowths. In view of the usual correspondence between the number of thallodic lobes present and the number of conidial chains existent most of the time, it is somewhat surprising to find on closer examination that often a lobe has two primary hyphal outgrowths, and that frequently a hyphal outgrowth bears one to three tips or spurs in addition to the one tip or spur supporting a conidial chain (FIG. 3, *G*). Indeed, the production of supernumerary parts often proceeds freely while material is directly under observation. A well developed thallus that at the time a mount is prepared shows only the usual four hyphal outgrowths (FIG. 3, *M*), each on a separate lobe, will often put forth a second outgrowth from each of the lobes during the ensuing hour. While such behavior may be held to result directly from artificial disturbance of the conditions under which the fungus had made its growth, rather similar disturbance must repeatedly have taken place, without human interference, in my cultures (as also in nature) from the jostling, for example, of robust nematodes. Assuredly in instances where thalli were represented only by empty membranous envelopes when the agar holding them was mounted for microscopical examination, the presence of four or five denuded spurs on one or more of the hyphal outgrowths (FIG. 3, *S, T*) should be considered expressive of normal morphology.

The protoplasm in the flat thallus shows a somewhat more granular structure than is usual in the helicoid thalli of *Cochlonema* and *Endocochlus*. In respect to the texture of its contents the flat thallus invites comparison, among the Zoöpagaceae, more especially, perhaps, with the filamentous mycelium of my *Stylopage hadra* (**4**). The migration of contents into the elongating aerial hyphae proceeds with increasing vacuolization in the thallus (FIG. 3, *G, b; N–R*) until only the empty desmid-like envelope remains visible within the empty protozoan pellicle (FIG. 3, *S, T*). Retaining walls similar to those laid down in some species of *Cochlonema* to mark successive stages of evacuation, have never been

seen in the fungus. Formation of conidia takes place here much as in *Cochlonema* and in the two other catenulate genera—*Bdellospora* and *Zoöpage*—earlier made known in the Zoöpagaceae. The flat pillowy thallus embodies a design for which no provision has hitherto been made in the family. A thallus of such outward shape would, of course, be quite commonplace among some groups within the Chytridiales, but among the Zoöpagaceae its departure from a filamentous condition seems no less unusual than that of the globose thalli developed in my *Bdellospora helicoides* (**2**). Accordingly the fungus would seem to merit recognition as the type of a separate genus. A generic name compounded of words meaning "unplaited" and "body," respectively, may be helpful in recalling its chief distinctive feature.

Aplectosoma gen. nov.

Corpora assumentia incolorata, intra animalia viventia praecipue intra Amoebas minores planas crescentia, subdiscoidea vel pulvinata, saepe mox in margine plus minusve lobulata, animali moribundo vel debilitato ex apicibus gibborum hyphas conidiferas emittentia; conidia hyalina, saepe fusoidea vel elongato-ellipsoidea, in catenulas erectas vel ascendentes digesta.

Assimilative thallus colorless, growing endoparasitically in small living animals, especially in rather small *Amoebae* of flattened shape, somewhat disc-shaped or cushion-shaped, at the margin eventually often becoming more or less lobulate, from the apices of the several lobules putting forth conidiiferous hyphae after the disablement or death of the animal; conidiiferous hyphae erect or ascending, at first continuous, later through partitioning becoming transformed into chains of conidia; conidia colorless, often spindle-shaped or elongate-ellipsoidal.

Aplectosoma microsporum sp. nov.

Corporibus assumentibus pulvinatis, in margine saepius quadrifidis vel interdum quinquifidis, plerumque 13–24 μ longis, 10–18 μ latis, ex 4 vel 5 gibbis hyphas fertiles emittentibus; hyphis fertilibus primo continuis, mox in sterigmate infero et catenula conidiorum supera constantibus; sterigmatis simplicibus vel aliquoties breviramosis, plerumque 4–15 μ longis, basi 1.5–3.5 μ crassis, sursum attenuatis, apice circa 0.5 μ crassis; conidiis in catenula erecta vel ascendente denis usque tricenis oriundis, fusoideis vel elongato-ellipsoideis, plerumque 3.5–5.2 μ longis, 1.4–1.9 μ crassis.

Amoebam speciem vulgo 20 usque 25 μ latam enecans habitat in foliis Salicis putrescentibus prope College Park, Maryland.

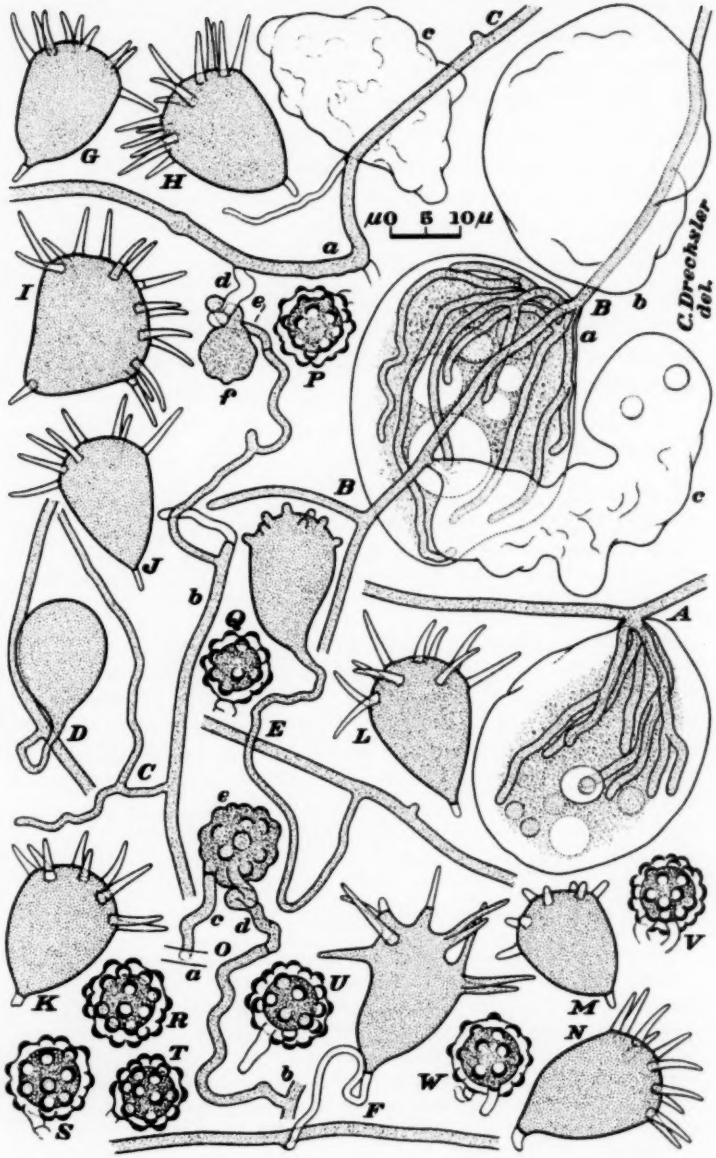


FIG. 4.

Assimilative thallus cushion-shaped, at the margin usually quadrilobate but sometimes quinquelobate, mostly 13 to 24 μ long and 10 to 18 μ wide, from the several lobes putting forth hyphae which later are converted individually into a basal sterigma and a distal, erect or ascending conidial chain; the sterigma simple or with several short spurs, mostly 4 to 15 μ long, 1.5 to 3.5 μ wide at the base, tapering upward, about 0.5 μ wide at the tip; the conidia spindle-shaped or elongate-ellipsoidal, mostly 3.5 to 5.2 μ long, 1.4 to 1.9 μ wide, formed in numbers from 10 to 30 in a chain.

Endoparasitic in an *Amoeba* mostly 20 to 25 μ wide, it occurs in decaying *Salix* leaves near College Park, Maryland.

THE SEXUAL REPRODUCTIVE STAGE OF ACAULOPAGE LOPHOSPORA

Acaulopage lophospora was originally described from an agar plate culture that had been planted with decaying vegetable material gathered in Colorado. The species at the time had never been observed in cultures prepared with vegetable detritus from other sources, though a closely related form, my *Acaulopage tetraceros* (**3**: 195–197; **9**: 289–291), would seem widely and abundantly distributed in Maryland and Virginia. More recently mycelium and conidia evidently referable to *A. lophospora* developed in a maizemeal-agar plate culture which after being overgrown by *Pythium ultimum* had been further planted with a small quantity of partly decayed honeysuckle (*Lonicera* sp.) leaves collected near Aberdeen, Maryland, on February 14, 1950. The mycelium (FIG.

FIG. 4. *Acaulopage lophospora* (Maryland strain), drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Portion of hypha with a captured *Amoeba* that has been largely expropriated of its protoplasmic contents by means of a rangy bush-like haustorium. *B*, Portion of hypha showing one captured *Amoeba*, *a*, extensively invaded by a rangy bush-like haustorium, and the empty pellicles of two other captured *Amoebae*, *b* and *c*. *C*, Two hyphae, *a* and *b*, without close mycelial connection; one of them, *a*, has attached to it the empty pellicle of a captured *Amoeba*, *c*; the two hyphae have produced two gametangia, *d* and *e*, respectively, which have united to put forth the young zygosporangium, *f*. *D*, Portion of hypha with a branch bearing a nearly full-grown conidium. *E*, Portion of hypha with a branch bearing a conidium that is putting forth distal protuberances. *F*, Portion of hypha with a branch bearing a full grown conidium whose protuberances are being evacuated of protoplasmic contents. *G–N*, Mature conidia. *O*, Immature unit of sexual reproductive apparatus: *a*, *b*, mycelial hyphae without close mycelial connection; *c*, *d*, gametangia; *e*, immature zygosporangium. *P–W*, Mature zygospores.

4, *A–F;* FIG. 5, *A*) here appeared, in general, a little coarser than in the earlier culture, some of the filaments attaining a width of 2 μ (FIG. 4, *C, a, b*). The *Amoebae* (FIG. 4, *A; B, a-c; C, c;* FIG. 5, *A*) captured in large numbers through adhesion to the mycelial filaments included individuals measuring as much as 35 μ in average diameter. In some of the captured animals a nearly spherical nucleus with a slightly darker central body could be recognized (FIG. 4, *A*). Expropriation of the animal's contents was accomplished by means of longish assimilative branches that arose in bush-like arrangement (FIG. 4, *A; B, a;* FIG. 5, *A*) from the lateral spur intruded by the external mycelial hypha through the adhering pellicle.

Conidia were produced freely by the Maryland fungus. During the earlier stages of development the lateral branch of somewhat variable length connecting the mycelial filament with the growing spore could often be made out clearly (FIG. 4, *D–F*). As a rule the young growing conidium showed a smooth obovoid contour (FIG. 4, *D*). In the final stages of enlargement it would burgeon forth most usually about ten tapering digitate protuberances from its upper side (FIG. 4, *E*). Once the protuberances were fully extended their protoplasmic contents were withdrawn backward. A cross-wall having in the meantime been laid down to delimit the conidium basally, the supporting branch was likewise evacuated of contents (FIG. 4, *F*). The completed conidium with its short empty stipe, and its crest of six to fifteen empty membranous appendages (FIG. 4, *G–N;* FIG. 5, *B–F*) corresponded well to the conidia produced by the Colorado strain of the fungus (FIG. 5, *G–J*).

In addition to forming conidia the Maryland strain gave rise to zygospores. Units of sexual reproductive apparatus regularly had their origin in two mycelial filaments (FIG. 4, *C, a, b; O, a, b*) without any close hyphal connection. From these filaments would arise two sexual branches which after becoming in some degree interlocked would each lay down a septum to delimit a terminal gametangium (FIG. 4, *C, d, e; O, c, d*). After apical fusion of the two gametangia a globose zygosporangium grew out close to the union (FIG. 4, *C, f; O, e*). During the later stages of their

growth the zygosporangia became covered with twenty to thirty verrucose protuberances. At maturity the protuberances appeared as handsome yellowish hemispherical warts, often about 1 μ high and 2 μ wide at the base. Owing to their presence the structure of the underlying wall was badly obscured, making it difficult to determine whether only a single thick layer surrounded the living protoplast, or whether a zygosporangial membrane enveloped a separate zygospore wall. The mature globose body (FIG. 4, *P–W*), exclusive of the warts, measured usually 9 to 11.5 μ in diameter; the spherical protoplast, mostly of granular texture, varied from 6.5 to 8.7 μ in that dimension.

A ZOÖPAGACEOUS MYCELIUM FOUND IN TESTACEOUS RHIZOPODS

Aplectosoma microsporum provides an example wherein the shape of the assimilative thallus is adapted in a distinctive manner to the spacial limitations of the host animal. Adaptation to somewhat different spacial limitations is shown in the curious disposition of a mycelium, evidently referable to the Zoöpagaceae, that was observed most often in a circular shield-shaped *Arcella* (FIG. 2, *K*; FIG. 5, *K*; FIG. 6, *A, B*), which from its close resemblance to two small specimens of *Arcella discoides* Ehrenb. figured by Leidy (**13**: *pl. 28, figs. 24–27*) I am referring to Ehrenberg's species. The infected animals were found among a much larger number of healthy individuals of the same species in maizemeal-agar plate cultures which after being overgrown with mycelium of *Pythium ultimum* had been further planted in some instances with small quantities of decaying crab grass (*Digitaria sanguinalis* (L.) Scop.) taken from an old weed pile on moist ground in College Park, Maryland, on March 26, 1950, and in other instances with small quantities of overwintered sedge detritus gathered nearby on the same day. Infection must almost certainly have taken place before the decaying material was added to the cultures, for the endoparasitic mycelium was always seen in a declining rather than in a growing condition. Within the shield-like testa the mycelium was disposed circularly, after the manner of a garland, between the central aperture and the peripheral margin. In specimens where the proximal hypha was clearly visible, even if in part evacuated

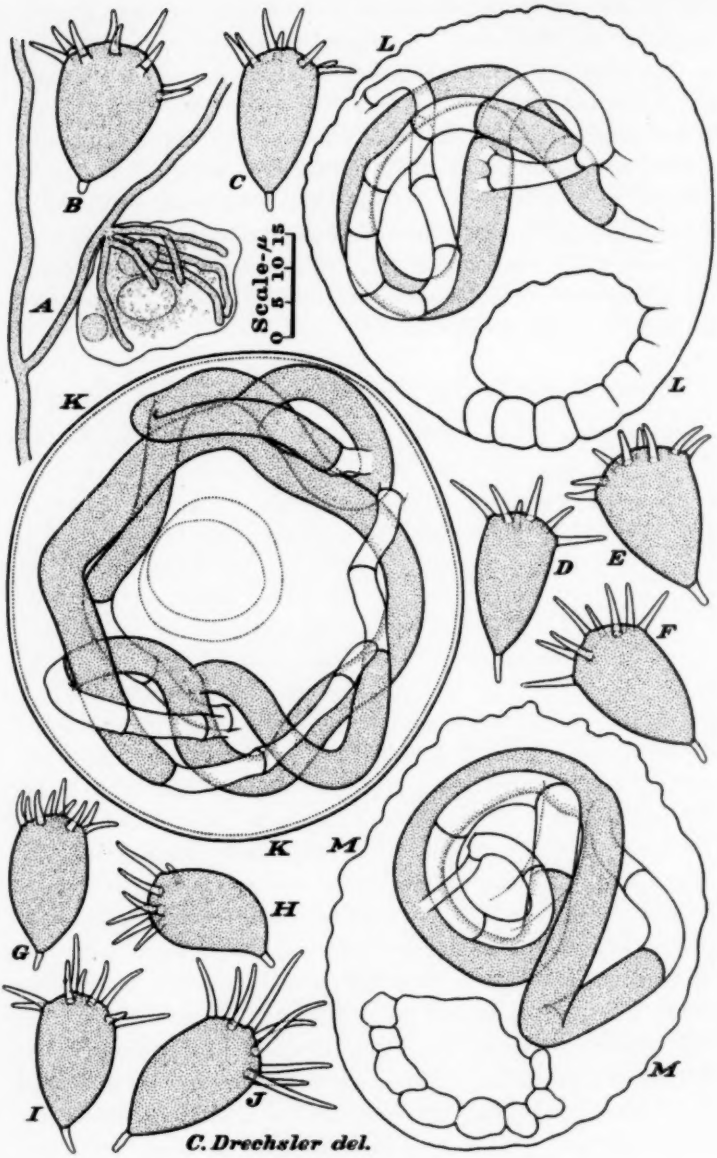


FIG. 5.

of protoplasmic contents (FIG. 2, *K*; FIG. 5, *K*), the fungus manifestly had begun its vegetative development as a filament between 2 and 3 μ thick. As it elongated it widened gradually, and thus usually attained a diameter of 6 to 7.5 μ on reaching a length of 75 to 100 μ, when it branched dichotomously. The two branches evidently grew for distances of 50 to 100 μ (FIG. 2, *K*; FIG. 5, *K*), or even for a distance of 150 μ (FIG. 6, *B*), before a second bifurcation occurred. Between the first and second dichotomies the branches maintained a rather uniform diameter, but beyond the second dichotomy the width of all hyphal branches diminished perceptibly. A third dichotomy was sometimes observed (FIG. 2, *K*), with the resulting ramifications measuring only about 2 μ in thickness. At the time the fungus came under observation the proximal hypha as well as the distal branches in all the mycelia was already emptied rather extensively of protoplasmic contents. Evacuation had evidently proceeded step by step, for the empty portions of hyphal membrane revealed a succession of cross-walls similar to the retaining walls bounding the filamentous living cell at its several ends. In one testa (FIG. 6, *A*) two separate living hyphal cells were found present—a condition that may have come about through plural infection. The protoplasm in the living portions of mycelium had the dense, somewhat homogeneous appearance familiar in the helicoid thalli of *Cochlonema* and *Endocochlus*.

Many of the cultures that contained infected specimens of *Arcella discoides* showed mycelia of the same fungus present also in the shells of another testaceous rhizopod (FIG. 5, *L*, *M*), which would seem referable to *Difflugia constricta* Ehrenb., judging from resemblances in shape and size to some individuals of that species figured by Leidy (**13**: *pl. 18, figs. 17–19*). In the more nearly globose chamber of this animal the individual mycelium formed a loose irregular clew without noticeable helicoid convolvement. Ex-

FIG. 5. Drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A–J*, *Acaulopage lophospora*: *A*, Portion of mycelium of Maryland strain, to which is attached a captured *Amoeba* that has been nearly depleted of its protoplasmic contents by means of a bush-like haustorium. *B–F*, Mature conidia of Maryland strain. *G–J*, Mature conidia of Colorado strain. *K–M*, Mycelial stage of endoparasitic zoöpagaceous fungus: *K*, Testa of *Arcella discoides* containing the fungus. *L*, *M*, Fungus present in testae of *Difflugia constricta*.

tensive evacuation of hyphal portions here likewise had resulted in deposition of successive partitions similar to the retaining walls bounding the living filamentous cell at the ends (FIGS. 5, *L, M*).

As progressive evacuation of mycelium or thallus in the Zoöpagaceae is commonly associated with formation of conidia or zygospores, the fungus in the two species of rhizopods was examined closely for reproductive development. No reproductive structures of any sort could be recognized. In instances where the proximal portion of the mycelium was visible, no empty spore envelope could be seen from which vegetative growth could have proceeded. Since empty shells of *Arcella discoides* sometimes contained a coiled eelworm, the possibility was considered that the fungus might be parasitic on the nematode visitors rather than on the protozoans themselves. The possibility seems a very unlikely one, inasmuch as development within imprisoned eelworms would not have permitted the rather broad garland-like hyphal arrangement often observed (FIG. 2, *K*; FIG. 6, *A*); and besides, no eelworms were ever found occupying empty shells of *Diffiugia constricta*. Material showing asexual reproduction will be necessary before the fungus can be assigned to an appropriate genus in the family.

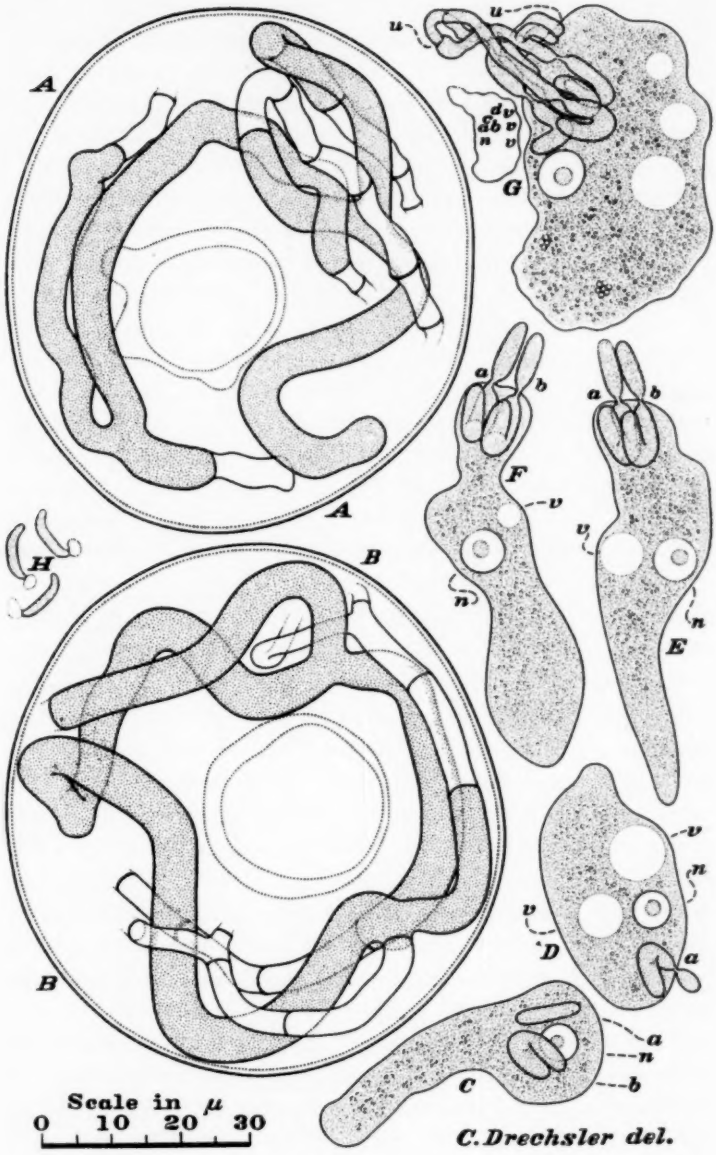
AN AMOEBA PARASITE WITH A SMALL U-SHAPED THALLUS

A Petri plate culture of rather soft maizemeal agar, which, after being permeated with mycelium of my *Pythium arrhenomanes*, had been further planted with a small quantity of barley (*Hordeum vulgare* L.) straw gathered near Greeley, Colorado, in October, 1945, showed on examination 18 days later scattered individuals of a species of *Amoeba* that harbored the smallest endoparasitic thallus hitherto seen in any member of the Zoöpagaceae. The *Amoeba* in question (FIG. 6, *C–G*) may have been of approximately the same size as the species parasitized by *Aplectosoma microsporum*, but was usually of more elongate shape. When in active locomotion it stretched out (FIG. 6, *E, F*) after the manner of *A. limax* Dujardin, though it lacked the curious tuft of posterior processes familiar in that species. In a fully extended condition the animal often measured 50 to 60 μ in length, while its width varied from 5 to 15 μ at different places along its body. It con-

tained a globose or somewhat prolate ellipsoidal nucleus 5.5 to 7 μ in diameter, with a slightly darker subspherical endosome 1.9 to 2.7 μ wide (FIG. 6, *C–G: n*). A single contractile vacuole (FIG. 6, *E, v; F, v*) was usually found operating in a peripheral position, and sometimes two (FIG. 6, *D, v*) or even three (FIG. 6, *G, v*) such vacuoles could be distinguished.

Infected specimens of the *Amoeba* showed fungous bodies of two types immersed in their protoplasm, a straight cylindrical type (FIG. 6, *C, a*) and a strongly curved, semi-circular or U-shaped type (FIG. 6, *C, b*). The straight bodies were often about 8 or 9 μ long and measured approximately 2.3 μ in greatest width; whereas the curved bodies varied commonly from 12 to 18 μ in length, and from 2.5 to 3.5 μ in greatest width. Many of the curved bodies were found connected at one of their two ends by a slender isthmus to an external body which in an early stage of development was of ovoid shape (FIG. 6, *D, a*), but later showed approximately the same dimensions and the same cylindrical or strongly elongated ellipsoidal shape (FIG. 6, *E, a, b; F, a, b*) as the straight bodies immersed in the protoplasm (FIG. 6, *C, a*). Although the curved bodies are small in comparison with the spirally convolved thalli usual in species of *Cochlonema* and *Endocochlus,* they manifestly are of the same character with respect to both function and form. The external bodies would seem best interpretable as conidia that are supplied with protoplasm by the curved thalli within the animal host. As not more than one presumptive conidium was ever seen attached to a thallus, there is reason to believe that each conidium on reaching definitive size becomes disjointed before its successor is burgeoned forth. It seems rather unlikely that only one single conidium is produced, for the thalli that are found connected with full grown conidia (FIG. 6, *F, a, b*) ordinarily show no sign of vacuolization as long as the animal host affords ample nourishment.

The manner in which the fungus gains entrance into the animal was not observed. Nor was conidial germination—at least germination of any easily recognizable sort—ever seen either inside or outside of host animals. From the similarity of fully grown conidia to the straight cylindrical bodies seen immersed in the host proto-


A
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Scale in μ
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FIG. 6.

plasm, it seems not improbable that the conidia are ingested by the *Amoeba*, and that they then develop directly into thalli through slight widening, strong curvature, and mere two-fold increase in length. Such immediate enlargement would offer some parallelism to the development of *Bdellospora helicoides*, where the externally adhering conidium expands rather directly into an obese thallus, though emission of the haustorium in that fungus provides a phase of growth somewhat akin to ordinary germination in species of *Cochlonema* and *Endocochlus*.

In some instances where an animal contained several curved thalli (FIG. 6, *G*, *a–d*) huddled close together, zygospores were produced. Each thallus would extend from one of its ends a narrow process which after perforating the host pellicle widened out externally into hyphae about 2.2 μ in diameter. Some of the hyphae made direct apical contact with one another in pairs (FIG. 6, *G*, *b*, *d*), while others became paired through tip-to-tip contact of branches put forth by them (FIG. 6, *G*, *c*, *d*). At the places of contact (FIG. 6, *G*, *u*) fusion occurred after cross-walls had been laid down to delimit gametangia. A verrucose zygospore about 6 μ in diameter resulted from each union. The several zygospores produced from plural thalli huddled in an animal formed handsome clusters outside, close to the pellicle. In mature clusters the continuity of the individual zygospores with the intertangled empty membranes of gametangia and supporting hyphae was found exceedingly difficult to follow.

FIG. 6. Drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, *B*, Mycelial stage of endoparasitic zoöpagaceous fungus: *A*, Two separate portions of living mycelium present in testa of *Arcella discoides*. *B*, Portion of living mycelium in testa of *Arcella discoides*. *C–G*, Zoöpagaceous fungus with a small curved thallus parasitic on a small *Amoeba* apparently of the *limax* type: *C*. *Amoeba* host containing a straight fungus body, *a*, perhaps representing a newly ingested conidium, as well as a curved thallus, *b*; *n*, host nucleus. *D*, *Amoeba* host containing a curved thallus, *a*, that is producing a conidium externally; *n*, nucleus of host; *v*, contractile vacuole of host. *E*, *F*, *Amoeba* host shown in two views drawn 15 minutes apart: *a*, *b*, two curved thalli, each of which has nearly completed production of a conidium externally; *n*, host nucleus; *v*, contractile vacuole of *Amoeba* host. *G*, *Amoeba* host containing four curved thalli, *a–d*, that have extended sexual hyphae which have made close contact in two positions, *u*; *n*, host nucleus; *v*, contractile vacuole of animal host. *H*, Three conidia of unnamed species of *Euryancale*.

Among the Zoöpagaceae that have hitherto become known the fungus is remarkable for the small size of its curved thallus, and the relatively large size of its conidia. The development of these conidia singly, and, in all likelihood, successively, makes its asexual reproduction different from any hitherto recognized in the family. There is reason to believe that the perseverance of conidial development through a long period when the animal host keeps on moving about actively, is a feature more consistent with an aquatic than with a terrestrial mode of life. In respect to habitat, therefore, the fungus could perhaps be placed in a category with the filamentous appendages observed on some aquatic rhizopods by different authors, including Korotneff (**12**), Leidy (**13**: 67–72), Penard (**14**: 65–70), Dangeard (**1**), and Geitler (**11**). While Leidy's figures of the appendages on his *Ouramoeba botulicauda* (**13**: *pl. 9, figs. 13–17*), and Geitler's figures of filaments attached to *Amoeba proteus* are suggestive of the Zoöpagaceae, catenulate sporulation would seem indicated in the illustrations of both authors rather than the solitary sporulation shown in my fungus.

A SECOND SPECIES OF EURYANCALE SUBSISTING ON NEMATODES

A maizemeal-agar plate culture which after being overgrown with mycelium of *Pythium ultimum* had been further planted with a small quantity of leaf mold taken from deciduous woods near Butternut, Wisconsin, on November 15, 1945, showed 25 days later a small area occupied by conidial apparatus of a species of *Euryancale* different from my *E. sacciospora* (**8**: 406–411). Its conidia (FIG. 6, *H*) consisted individually of a slightly curved cylindrical living cell usually from 7.5 to 9 μ long and about 1.5 μ wide, together with an empty basal appendage of prolate ellipsoidal shape commonly measuring 2 to 3 μ in length and approximately 1.8 μ in width. The appendage was oriented most often with its longer axis at a right angle to the proximal portion of the living cell. Unlike the empty appendage of *E. sacciospora* its saccate shape was not modified by conspicuous narrowing near the place of attachment. The upcurved lateral branches bearing the spores seemed markedly sturdier than the corresponding branches of *E.*

sacciospora. Further, the main reproductive hyphae giving off the upcurved branches appeared about twice as wide as the homologous hyphae of *E. sacciospora*.

Although several of the reproductive filaments could readily be traced backward to their origin in an assimilative mycelium within the integument of an eelworm, the remains of the eelworm were too largely concealed from view by opaque overlying material to permit identification of the host animal. No further development of the fungus could be noted during ensuing weeks, either because the host animal failed to multiply in the culture or because conditions were unsuitable for infection.

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THE RUSTS OF ARMERIA AND LIMONIUM IN NORTH AMERICA [1]

D. B. O. Savile [2] and I. L. Conners [3]

The rusts of *Armeria* and *Limonium* in North America were originally treated by Arthur (**1**) as a single species, *Uromyces Limonii* (DC.) Lév., or, to use the terminology adopted in the North American Flora, *Nigredo Limonii* (DC.) Arth. Later Arthur (**2**) recognized the rust of *Armeria* to be distinct and treated it under the name *Nigredo Armeriae* (Schlecht.) Arth. (*Uromyces Armeriae* (Schlecht.) Lév.). Finally (**3**) he restored the name *Uromyces Limonii* and treated the rust of *Armeria* as a variety of the species, making the combination *Uromyces Limonii* (DC.) Lév. var. *Armeriae* (Schlecht.) Arth.

Several years ago, the junior author noticed that the rust on *Limonium carolinianum*, which is abundant on the Atlantic coast and in the Gulf of St. Lawrence, appeared to lack uredinia. Further collections made throughout the season confirmed this lack, and detailed studies showed other small differences. More recently, the discovery of *Uromyces Armeriae* on the coast of Hudson Bay led to the recognition of geographic variation within this species and of further differences between it and *U. Limonii*. It now seems appropriate, therefore, to review the relationship and distribution of these rusts.

Uromyces Armeriae and *U. Limonii* certainly are closely related, as is indicated by the morphology of the peridial cells and urediniospores. In particular, the close-set, short and rather broadly conic ornamentations of the urediniospore walls are distinctive. These ornamentations may be regarded as either verrucae or echinulations, but close examination shows them to taper to a point and the writers accordingly prefer the latter term. Arthur (**3**) distin-

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guished the *Armeria* rust from the rust on *Limonium* only by the thicker urediniospore walls of the former and its broader teliospores with fragile pedicels. Measurement of a variety of specimens has shown that, in *U. Armeriae,* the aeciospores and peridial cells are consistently shorter and the markings of the peridial cells appreciably finer than in *U. Limonii.* Furthermore, there is generally a sporadic germination of the teliospores of *U. Armeriae* throughout the growing period, and consequently aecia are often found simultaneously with uredinia and telia. In *U. Limonii,* teliospore germination and the production of aecia seem to be confined strictly to the spring. Aecia of *U. Limonii* are actually seldom seen in herbarium specimens, many of the collections so labelled being aecia of the unreported rust on *Limonium carolinianum.*

As already indicated, it has been found that geographic variation exists within *Uromyces Armeriae,* and it is necessary to choose a taxonomic category for the variants. Most mycologists and plant pathologists reserve the term variety, in the parasitic fungi, to forms that show host specialization accompanied by minor morphological distinctions. This view was accepted by Arthur (**3**), who defined variety as "a more or less marked morphologic variant of species, associated with a different selection of hosts, and arising from aecial or telial infection." In *Uromyces Armeriae,* three morphological variants have arisen apparently by geographic isolation rather than host specialization. All the specimens examined have been on what Lawrence (**5**, **6**) showed to be mere varieties of a single cosmopolitan thrift, *Armeria maritima.* Lawrence (**6**) further showed that some of these varieties intergrade at the junctions of their ranges, and two rust specimens seen from British Columbia are, in fact, on plants intermediate between the varieties *purpurea* and *californica.* There is no reason to suppose that these minor host differences have been concerned in the morphological variation seen in *Uromyces Armeriae,* whereas the wide geographic separation of the variants is ample to account for their differences. It seems undesirable to use, for variants that have arisen by geographic isolation, the term variety, which is now well established in its application to parasites that have diverged because of host specialization. Varieties in this sense commonly occupy the same

or overlapping geographic ranges. It is accordingly proposed to use the term subspecies for these geographic variants, although doing so is not strictly in accord with the concept of a subspecies as a category intermediate in magnitude between a species and a variety. In support of this procedure the following points are cited: (1) Practically no use has been made of subspecies in the microfungi, and little even in the macrofungi. (2) The concept of species in the microfungi seldom permits the recognition of two lesser categories with different degrees of morphological variation; thus distinction between subspecies and varieties based on degrees of variation would be even more uncertain than it is in the higher plants. (3) The restriction of the term variety in the parasitic fungi to variants resulting from host specialization has been widely accepted, although it departs as much from the orthodox view as does the proposed use of the term subspecies. (4) Increasing recognition of geographic variants within clearly defined species is to be expected as they are examined more critically. The writers prefer to adopt the term subspecies rather than to add a completely new term to a heavily burdened system of nomenclature.

The following key is provided for the species under study.

1. Aeciospores 18–27(–29) μ long; peridial cells 22–37(–41) μ long; teliospore pedicels fragile.....*Uromyces Armeriae* sspp.
   2. Urediniospore pores 2–3; teliospore walls abruptly thickened at apex.....*U. Armeriae* ssp. *Armeriae*
   2. Urediniospore pores (2–)3–4(–5); teliospore walls abruptly thickened at apex.....*U. Armeriae* ssp. *hudsonicus*
   2. Urediniospore pores 2–3(–4); teliospore walls thickening gradually from base to apex.....*U. Armeriae* ssp. *pacificus*
1. Aeciospores 21–34 μ long; peridial cells 26.5–45(–53) μ long; teliospore pedicels firm.
   3. Uredinia lacking; teliospore pedicels to 110 μ long; some teliospores germinating during current season.....*U. Limonii-caroliniani*
   3. Uredinia present; teliospore pedicels to 85 μ long; teliospores never germinating during current season.....*U. Limonii*

UROMYCES ARMERIAE (Schl.) Lév.

Uromyces Armeriae is widely distributed in Europe and North America on several varieties of *Armeria maritima*. Sydow and Sydow (8) also report its occurrence on *A. juniperifolia* var.

splendens (*A. splendens*), and *A. plantaginea* var. *longibracteata* (*A. longibracteata*); but the nomenclature of *Armeria* was until recently so confused that such records cannot be accepted unreservedly. The material examined from three widely separated regions allows recognition of the following subspecies.

UROMYCES ARMERIAE (Schl.) Lév. *ssp.* **Armeriae** *nom. nov.*

Pycnia amphigenous. Aecia amphigenous, cupulate, in small groups. Aecial stage not seen, probably indistinguishable from other subspecies. Description of peridial cells and spores from Klebahn (**4**): Peridial cells very variable in shape, to 44 μ long and 22 μ wide in almost regular rows; outer wall 5–10 μ, inner wall 2–3 μ thick, finely warted. Aeciospores 17–26 (to 33 according to Bubák) × 17–22 μ, rounded polyhedric, isodiametric or elongate; wall thin, very closely and finely warted. The following description of the uredinia is based on a specimen in the Mycological Herbarium of the Division from Sweden on *Armeria maritima* var. *elongata*, and Roum. F. Sel. 2632, J. Kunze F. Sel. 33 and All. & Schn. F. Bav. 604 all on *A. maritima* (perhaps var. *purpurea* but specimens inadequate for host determination). Uredinia amphigenous or caulicolous, scattered. Urediniospores (22.5)–24–33 × 20–27 μ; wall 1.5–2.6 μ thick, yellowish brown, thickly covered with broadly conical spines at 0.4–1.0 μ intervals; pores 2–3 equatorial or scattered. Telia, on basis of All. & Schn. F. Bav. 604 and the Swedish specimen mentioned above, amphigenous or caulicolous, scattered. Teliospores 25–33 × 22–29.5 μ; wall 1.5–2.5 μ at sides, chestnut, smooth, abruptly thickened at apex to 4–6 μ including a yellowish brown umbo; pedicel hyaline, fragile.

Uromyces Armeriae was described from Berlin on *Armeria maritima* var. *purpurea* (*A. vulgaris*). The three exsiccata referred to above may therefore be assumed to be authentic material of the typical subspecies. It is possible that a slightly different form occurs in northern Europe, since, in the Swedish specimen examined, the urediniospore pores were usually two, rather than equally two or three; but the distinction is not believed to be important.

UROMYCES ARMERIAE (Schl.) Lév. *ssp.* **hudsonicus** *ssp. nov.*

Pycnia rara, inconspicua. Aecia amphigena, gregaria vel sparsa. Cellulae peridii 22–31 × 17–25 μ, pariete externo subtiliter undatim striato, interno verrucoso. Aeciosporae 18–24 × 16–22 μ; episporio hyalino, c. 0.5 μ crass., minute verrucoso. Uredinia sparsa, amphigena vel rarius caulicola. Urediniosporae 23–29.5 × 21–25 μ; episporio 1.5–2.5 μ crass., ochraceo, dense echinulato, (2)–3–4–(5) poris in circulo aequatoriali vel sparsim instructo. Telia sparsa, amphigena vel rarius caulicola. Teliosporae 23.5–34.0 × (16.5)–19.0–23.5 μ; episporio castaneo, leve, latere 1.5–2.5 μ crass., summo 3.0–5.0 μ abrupte spissato; pedicello hyalino, fragili.

In foliis caulibusque *Armeriae maritimae* var. *labradoricae*, prope Flumen Magnum Balaenarum, Canada.

On *Armeria maritima* var. *labradorica*, Great Whale River, Quebec, Canada, 15 July 1949 (DAOM 23450) TYPE; 3 Aug. 1949 (DAOM 23451) TOPOTYPE. Type in Mycological Herbarium, Division of Botany and Plant Pathology, Science Service, Dept. of Agriculture, Ottawa. Co-type material in Arthur Herbarium, Purdue University; Farlow Herbarium, Harvard University; University Herbarium, University of Michigan; Division of Mycology and Disease Survey, U.S.D.A., Beltsville, Md.; Dept. of Botany, University of Toronto; Commonwealth Mycological Institute, Kew; Dept. of Botany, University of California, Berkeley; Dept. of Botany, University of Wisconsin; Naturhistoriska Riksmuseet, Stockholm. Remaining co-type material and topotype material to be contributed to Lund Botanical Exchange Club.

Armeria maritima var. *labradorica* is abundant on the raised sand delta at Great Whale River, on the east coast of Hudson Bay. Two rusted colonies were found in this area by the senior author in 1949. This rust differs from the European form in having slightly smaller urediniospores with generally three or four pores. Although further collecting may eventually provide specimens intergrading both with the European rust and with that on the Pacific coast, these considerable differences seem worthy of recognition. It is a point of some interest to note that only two groups of pycnia could be found in the type collection although aecia were abundant on most plants. The life history of *U. Armeriae* is plainly in need of further study.

UROMYCES ARMERIAE (Schl.) Lév. *ssp.* **pacificus** *ssp. nov.*

Pycnia ignota. Aecia amphigena, gregaria vel sparsa. Cellulae peridii (19)–24–37–(41) × 15–25–(28) μ, pariete externo subtiliter undatim striato, interno verrucoso. Aeciosporae 19.0–26.5–(29.5) × 17–23 μ, episporio hyalino, 1.0–1.6 μ crass., minute verrucoso. Uredinia sparsa, amphigena vel rarius caulicola. Urediniosporae 26.0–34.5 × 22.0–30.0–(31.5) μ; episporio ochraceo, 1.8–2.8 μ crass., dense echinulato, 2–3–(4) poris in circulo aequatoriali vel sparsim instructo. Telia sparsa, amphigena vel rarius caulicola. Teliosporae 25.0–34.0–(36.5) × 19.0–27.0 μ; episporio castaneo, levi, baso (1.0)–1.5–2.0 μ crass., ad apicem 4.5–7.0 μ gradatim spissescente; pedicello hyalino, fragili.

In foliis caulibusque *Armeriae maritimae*, California, Oregon et Columbia Britannica.

On *Armeria maritima*, intermediate between vars. *californica* and *purpurea*, Saanichton, B. C. (DAOM 5627, TYPE, and DAOM 5521); on *A. maritima* (cult.), Victoria, B. C. (DAOM 19191); on *A. maritima* var. *californica*, Ore. (H. S. Jackson 3018) and Calif. (Arthur Herb. 16913, 16915). The type specimen bears aecia, uredinia and telia; the collection is not large enough for wide distribution, but a fragment is being deposited in the Arthur Herbarium. Neither this specimen nor 16913 from the Arthur Herbarium, which bears aecia only, show any sign of pycnia. The occasional occurrence of uredinia or telia in aecial sori suggest either that some of the aecia are secondary, or that the life cycle is unstable and is becoming reduced and that homothallism may have been already attained.

The rust from the Pacific coast produces urediniospores equal in size to those of the European rust or slightly larger and with two to four pores. Most of the teliospores are globoid and with the wall gradually increasing in thickness from base to apex, rather than being abruptly thickened at the apex as in the other two subspecies. A further minor difference is that Pacific coast specimens tend to have slightly thicker urediniospore walls than those from elsewhere, which may have been the basis of the statement by Arthur (**3**) that in the *Armeria* rust the urediniospore walls are thicker than in that on *Limonium*.

UROMYCES LIMONII (DC.) Lév.

Uromyces Limonii occurs naturally in the United States on *Limonium californicum* in California, and on *L. limbatum* in New

Mexico and Texas. It is also reported by Weiss (**9**) on the cultivated *L. latifolium* in Connecticut. The only records of its occurrence in Canada are on *L. latifolium* in British Columbia and *L. vulgare* in Ontario. The only native *Limonium* in Canada is *L. carolinianum*, which is abundant in the Gulf of St. Lawrence and along the Atlantic coast; but the rust on this species, as already indicated, is not *U. Limonii*.

The teliospores of *U. Limonii* evidently germinate promptly in the spring and never at any other season. Consequently aecia appear less frequently in specimens of this rust than of *U. Armeriae* or *U. Limonii-carolinianae*. In the following description the dimensions of the aeciospores and peridial cells are based on material on *Limonium californicum* and *L. limbatum*. The description of the urediniospores and teliospores is based on material from all the North American hosts listed above and from *L. Gmelini*, Bavaria (Linh. F. Bav. 211), and *Limonium* sp., Sweden. All this material agrees fairly closely except that the urediniospores on *L. californicum* are usually appreciably larger than those from other hosts and other localities. The range of sizes on *L. californicum* is 28–36.5–(40.5) × (21)–24–30–(31) μ. In the other specimens examined it is 23–33–(34.5) × 20–28–(30) μ. However, no other appreciable distinctions occur and considerable variation in urediniospore size is found between individual collections. It, therefore, seems inadvisable at present to treat the California rust as a distinct entity. A composite description of the species follows.

Pycnia amphigenous. Aecia amphigenous, cupulate, in groups. Peridial cells 30–53 × 16–29 μ; outer wall finely sinuate-striate, more conspicuously so than in *U. Armeriae*, inner wall strongly verrucose. Aeciospores 22–34 × 19.5–27 μ; wall 0.8–1.8 μ, minutely verrucose, hyaline. Uredinia amphigenous. Urediniospores 23–36.5–(40.5) × 20–31 μ; wall 1.2–2.2 μ, yellowish brown, closely echinulate; pores 2–3–(4) equatorial or scattered. Telia amphigenous. Teliospores 24–42–(46.5) × 16–26–(28.5) μ; wall 1.0–2.0–(2.7) μ at sides, increasing, often abruptly, to 4.0–7.5–(9.5) μ at apex, chestnut, smooth; pedicel hyaline to pale yellow, firm, up to 80(86) μ long.

Uromyces Limonii-caroliniani *sp. nov.*

Pycnia amphigena. Aecia amphigena, gregaria. Cellulae peridii 26.5–45 × 16–30 μ, pariete externo leviter undatim striato, interno fortiter verrucoso. Aeciosporae 21–33 × 19–27–(30) μ; episporio hyalino, 1.0–1.8 μ crass., minute verrucoso. Uredinia non efficiuntur. Telia amphigena vel rarius caulicola. Teliosporae (24.5)–28–46.5–(50) × 16.5–27.5–(29) μ; episporio castaneo, levi, latere 1.0–3.0 μ crass., summo (3.5)–5.0–8.0–(9.5) μ abrupte spissato; pedicello persistenti, subhyalino vel flavido, usque 110(120) μ long.

In foliis caulibusque *Limonii caroliniani.*

Many specimens examined show very little variation from one another. The TYPE, DAOM 18686, collected by Mr. A. Payette at Rivière Ouelle, Que., 23 June 1946, has been selected because it shows all spore stages, is in good condition and is thoroughly typical; portions are being deposited in the Arthur Herbarium and the Commonwealth Mycological Institute. Specimens examined include F. Columb. 649, 658, 2294 and 3096, Rel. Farl. 297, and several others from Quebec, New Brunswick, Nova Scotia and Mississippi.

All aecia examined, even in F. Columb. 658, which was collected in September, are accompanied by pycnia. Presumably, therefore, all aecia are primary, since secondary aecia would be expected to lack pycnia. The appearance of aecia throughout the summer may be due partly to delayed germination of the teliospores of the previous season; but sparing germination of the current season's spores was observed in F. Columb. 2294 and 3096 and in DAOM 19648 (Bic, Que.), all collected in August.

The main distinctions of this species from *U. Limonii* are its lack of uredinia and the occurrence of aecia at any period in the growing season. Further minor differences are a preponderance of decidedly elongate teliospores and a greater maximum pedicel length in *U. Limonii-caroliniani.*

Two other described species required consideration as being possibly identical with *U. Limonii-caroliniani.* In the description of *U. guayacuru* Speg. only aecia and telia are mentioned. Dr. J. C. Lindquist, Instituto de Botanica Spegazzini, states (*in litt.*) that *U. guayacuru* agrees exactly with the eu-autoecious rust *U. Limoni* from Europe and North America. A specimen on *L. brasiliense,* received from Dr. Lindquist and stated to have been checked with

the type of *U. guayacuru*, confirms this view both by the presence of uredinia and in the length of the teliospore pedicels. Accordingly *U. guayacuru* is distinct from *U. Limonii-caroliniani*.

Uromyces Staticae-sinensis Liou & Wang (**7**) (*U. Statices-sinensis* nom. emend.) was described as producing aecia and telia on *Limonium sinnense* at Chefoo, China. Since the original description is not widely available, the English diagnosis is herewith reproduced verbatim: "Aecidia amphigenous, gregarious into purplish spots, shortly cylindric, cup-like, whitish, edge torn; aecidiospores angularly globose or globose, yellowish white, about 16–19 μ. Teleutosori amphigenous, rounded, small, scattered, convex, somewhat waxy, naked, black; teleutospores globose to subglobose, very rarely shortly elliptic-oblong, thickened at the top, smooth, brown, 13–27 $\times$ 20–30 μ; pedicel up to 90 μ, persistent, light-brown." The otherwise identical Latin diagnosis gives the maximum teliospore width as 17 μ, but this is presumably a typographical error since the spores are stated to be largely globose. The type of this species has not been available for study, but a collection by Hiratsuka, on *L. sinense* and ascribed to this species, collected 3 Oct. 1942 at Kojyo, Kinshu Province, S. Manchuria, was recently received by the Arthur Herbarium. Study of this specimen reveals amphigenous telia; spores 23.5–34.5 $\times$ 17.5–25.5 μ; wall 1.5–3.0 μ near base, increasing, usually gradually, to 6.0–8.0 μ at apex, chestnut, smooth; pedicels usually less than 80 μ, rarely to 100 μ long, firm, subhyaline to pale yellow. A few collapsed urediniospores in the telia were 21–25 $\times$ 18.5–22 μ; wall 1.4–1.8 μ, yellowish brown, closely echinulate, pores apparently 2–3 and more or less equatorial but indistinct owing to state of spores. Although the teliospores in this specimen are slightly larger than those described for *U. Statices-sinensis*, the general agreement is good. *U. Statices-sinensis* seems to be distinct from both *U. Limonii* and *U. Limonii-caroliniani*. It differs from the former in evidently lacking uredinia, although forming a few urediniospores in the telia, and in having smaller teliospores with slightly longer pedicels; and from the latter in the occasional formation of urediniospores, and in having decidedly smaller and more globose teliospores with slightly shorter pedicels. *U. Statices-sinensis* and *U. Limonii-caroliniani* seem to have been derived independently from *U. Limonii*.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Mr. Albert Payette, who made a series of collections of *U. Limonii-caroliniani* in eastern Quebec to confirm the lack of uredinia in that species; to Dr. J. C. Lindquist for a specimen of *U. Limonii* on *Limonium brasiliense;* and to Dr. Geo. B. Cummins for advice, suggestions and the loan of specimens.

SUMMARY

Uromyces Armeriae is shown to be divisible into three well-defined geographic subspecies, ssp. *Armeriae* in Europe, ssp. *hudsonicus* on the coast of Hudson Bay, and ssp. *pacificus* on the Pacific coast of North America.

Uromyces Limonii-caroliniani, on *Limonium carolinianum,* is distinguished from *U. Limonii,* on other *Limonium* spp., in lacking uredinia, producing aecia throughout the summer, and having slightly longer teliospores and teliospore pedicels.

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THE GENUS CYTIDIA

WM. BRIDGE COOKE

(WITH 24 FIGURES)

Cytidia is a genus of fungi assigned by most contemporary American workers to the Thelephoraceae, by most contemporary European workers to the Cyphellaceae. The Cyphellaceae is a family which has been segregated from the Thelephoraceae because of the cup-shaped structure of the receptacle, which has definite margins, although margins of adjacent receptacles may become confluent. In general these fungi present the aspect of inverted Discomycetes.

To the Cyphellaceae are assigned such genera as *Cyphella, Solenia, Porothelium, Cytidia* and *Aleurodiscus.* Both *Cytidia* and *Aleurodiscus* usually have large basidia, large basidiospores, and distinctive paraphyses of an acanthophysoid nature, characters which are usually lacking in the other three genera, in whose hymenia sterile bodies rarely occur and which usually have small basidia and basidiospores. *Cytidia* is further differentiated in that the hyphae of its receptacles are usually strongly gelatinized. Because of these characters the present writer prefers to leave the genera *Cytidia* and *Aleurodiscus* in the Thelephoraceae without reference, at present, to any segregate of the family.

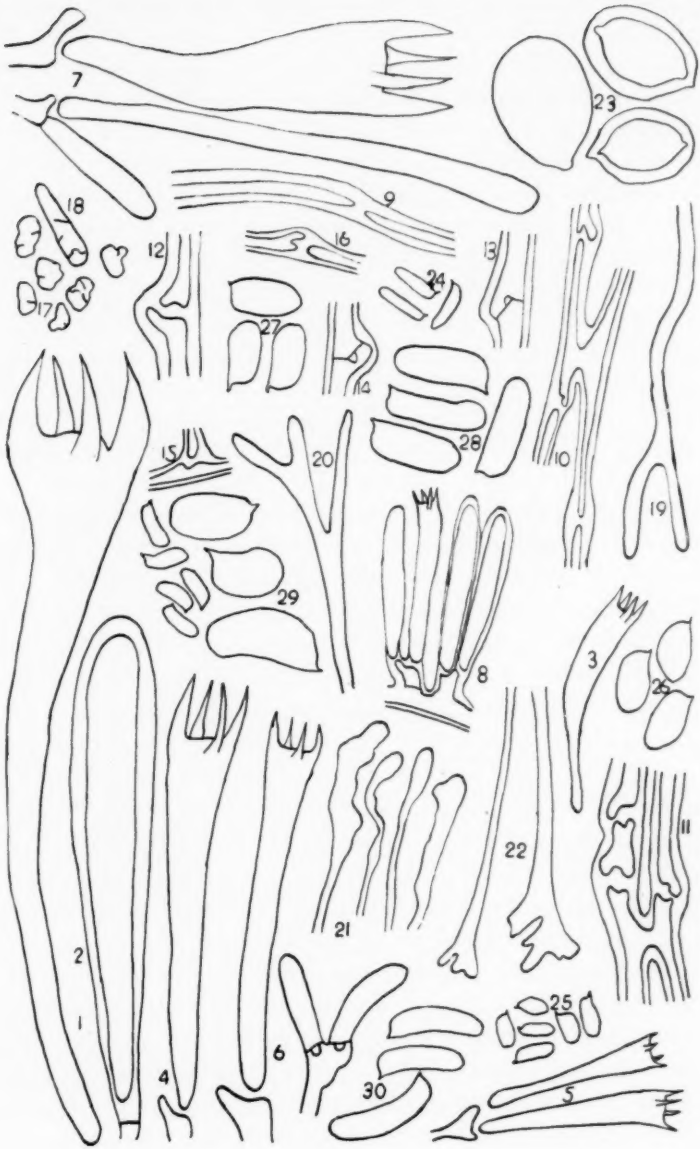
In *Cytidia,* four species have been commonly recognized. The most widespread of these is *C. salicina* which occurs on willows and other deciduous woody plants in the northern hemisphere. While not collected frequently, *C. flocculenta* is also widespread throughout the northern hemisphere and also occurs in Australia. *C. pezizoidea* and *C. habgallae* are widespread throughout the tropics of both hemispheres but collected infrequently. *C. simulans* was described from South Africa. Two species of restricted distribution will be described below; of these, one will be based on a collection from northern Idaho, the other on a few collections from northern California. Another species, usually placed in

synonymy with *C. salicina,* will be added to the genus based on collections from Latvia.

The anatomy of the receptacles of these species is relatively simple. The receptacle is attached by a central point to the substratum. The shape of the receptacle is round to oval and the point of attachment may be central or excentric. When a number of receptacles are growing in a small area on the under side of a branch their edges may meet and become fused. This fusion may include a large enough number of receptacles to give the appearance of a single widespread receptacle although on close observation the individual receptacles can be easily differentiated. In some cases this type of growth gives a merulioid aspect to the multiple fructification. The receptacles always appear on the under surface of the substratum, whether it be living or dead standing limbs or branches, or wood on the ground. Thus the hymenium is hypogenous. The edges of the receptacle are free and when dry they curl inward over the hymenium which they do not cover except in the case of small specimens. The upper surface of the receptacle, that which lies against the wood or which is exposed on drying, is usually covered with hairs. Between this hairy surface and the hymenium is the gelatinous context. In section this context is similar to that of some species of *Stereum.* It is composed of loosely to densely interwoven hyphae which are arranged more or less parallel with the substratum. In the area 50–100 μ below the hymenium there is a tendency for these hyphae to become oriented in such a position that they eventually become perpendicular to the substratum and then produce the hymenium. This tendency is more pronounced in some specimens than others, it is more pronounced in the two new western United States species than in other species, on the average.

In all specimens examined the subhymenial tissue is liberally supplied with clamp connections. In specimens examined of seven of the eight species to be discussed all the context tissue has clamp connections at each cell wall. In *C. flocculenta* hyphae were observed which branched at the clamp connections. In *C. lanata,* from Idaho, no clamps were observed in the context tissue.

In all specimens examined the context tissue was simple. It was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composed of a monomitic hyphal system all of whose hyphae terminated in hymenial or subhymenial elements on the one side and in surface hairs (when present) on the other. The hyphae near the hymenium were rather tightly interwoven while those near the upper surface were loosely interwoven. Consequently the gelatinously thickened walls of the hyphae near the hymenium were narrower, compared with the thickness of the lumen, than those of the hyphae near the upper surface. This type of thickening extended somewhat to the cross walls in the clamp connections which were correspondingly thicker toward the upper surface than toward the lower surface. This thickness made it impossible in usual smear mounts to see the protoplasmic connections between the cells and through the clamp.

The surface of the receptacles varies considerably between species. In *C. pezizoides, C. simulans* and a form of *C. salicina* the surface is smooth or nearly so. It is composed of little-differentiated cells of the context or in some cases it is formed of atro-sclerotized cells. In other species two types of surface covering were encountered. In *C. salicina, C. sarcoides, C. flocculenta,* and to a certain extent in *C. lanata, C. habgallae* and *C. stereoides,* this was composed of an irregular trichoderm made up of loosely interwoven, more or less parallel-arranged hyphae which continued

FIGS. 1, 2, 13, 19, 23. *Cyphella habgallae.*—1. basidium; 2. cystidium; 13. clamp-connection; 19. paraphysis tip; 23. two thick-walled mature basidiospores, one thin-walled immature basidiospore. FIGS. 3, 11, 29. *C. simulans.*—3. one four-sterigmate basidium; 11. H-piece in hypha with three clamp-connections; 29. three large basidiospores, five small basidiospores. FIGS. 4, 17, 18, 20, 30. *C. salicina.*—4. basidium; 17. conducting organ; 18. six chlamydospores; 20. paraphysis tip; 30. three basidiospores. FIG. 26. *C. sarcoides.*—26. three basidiospores. FIGS. 5, 9, 25. *C. lanata.*—5. two basidia; 9. piece of hypha without clamp-connection; 25. six basidiospores. FIGS. 6, 12, 22, 27. *C. pezizoides.*—6. mature basidium with two young basidia; 12. clamp-connection; 22. two paraphysis tips; 27. three basidiospores. FIGS. 7, 14, 21, 28. *C. stereoides.*—7. one short basidium, one young long basidium; 14. clamp-connection; 21. four paraphysis tips; 28. four basidiospores. FIGS. 8, 10, 15, 16, 24. *C. flocculenta.*—8. group of one mature basidium, one immature basidium and two thick-walled, sterile organs, bases of all four structures with gelatinous material confluent with that of parent hypha; 10. hyphal H-piece with three clamp-connections (two in face view, one in side view); 15. hyphal branch with gelatinously thickened septa; 16. clamp-connection; 24. three basidiospores.

beyond a more or less sclerotized region at the surface of the context as a white tissue. In macroscopic observation this gives rise to the pruinose surface of *C. salicina* and *C. sarcoides*, and to the flocculent surfaces of *C. flocculenta*, *C. habgallae* and *C. lanata*. In *C. stercoides*, and to a less extent in *C. habgallae* and *C. lanata*, this trichoderm becomes arranged in hyphal ropes which branch and anastomose. The ropes themselves may anastomose and individual hyphae may be found which connect one or more of these ropes. This produces a flocculent aspect on the surface of the receptacles; the surface is white in color. The hyphae of the trichoderms described above are thick-walled. The walls are hyaline and may be gelatinous. When the hyphae of these trichoderms are septate, clamps occur at the septa except in *C. lanata*. In all cases the trichoderm is separated from the context by a dark area in which some of the cells appear to be sclerotized. The trichoderm hyphae, however, are continuous with the hyphae of the context.

The receptacles of the preceding year may be found on branches in the current collecting period. However, these are usually sterile and the hymenium overgrown with hyphae. In two species the possibility of a perennial receptacle exists. In *C. lanata* there is a definite area separated from the hymenium by a region of normal context in which a palisade of tissue exists. This palisade contains the brown pigment associated with active hymenial tissues. The collapsed cells of this palisade agree in size and shape with basidia. Between these cells lie cells which connect the older or lower context with the newer or upper context. The two context areas are also connected by cells which may have been paraphyses in the first hymenium. It is thus possible to assume that in this specimen, at least, active growth and spore production took place in at least two seasons. In *C. simulans* the receptacle is definitely perennial as indicated by similar tissue arrangements in the hymenial and subhymenial regions.

The situation in *C. habgallae* remains uncertain. Especially in the area next to the substratum, and less frequently scattered throughout the context, are characteristic thick-walled basidiospores. Whether this indicates that as the context thickens through continued growth pushing the hymenium farther from the sub-

stratum mature spores are accidentally left behind the hymenium in the context, or whether the spores become scattered in these positions through the preparation of the specimen and through preparation of sections from it, is not clear. Ontogenetic studies of the development of the receptacles of this species will be necessary to disclose whether or not basidiospores whose walls have thickened in position on the basidium become too heavy for normal discharge and become overgrown in the developing receptacle. The spores are basidiospores, not of the *Matula*-type, so that the basidial receptacles are probably separate from the *Matula* receptacles of the imperfect stage, at least in material examined to date.

CYTIDIA QUÉLET, Fl. Myc. Fr. 25. 1888

Lomatina Karst. Finska Vet.-Soc. Bidr. Nat. o. Folk. **48**: 403. 1889.

Auriculariopsis R. Maire, Rech. Cyt. et Tax. 102. 1901.

Matula Massee, Jour. Roy. Micr. Soc. **1888**: 173. 1888.

Aleurocystus Lloyd (as McGinty), Myc. Writ. **6**: 1088. May, 1921.

Glocosoma Bres. *sensu* Lloyd, Myc. Writ. **6**: 1088. May, 1921.

Receptacles coriaceous to fleshy-gelatinous, cup-shaped, sessile, attached at a central point, scattered or crowded, often confluent; hymenium even at first, becoming somewhat wrinkled or veined in some cases; basidia simple; spores hyaline to yellowish, amyloid in Melzer's reagent.

Key to Species

1. Basidiospores becoming thick walled, sometimes embedded in the context; with imperfect receptacles assignable to the form genus *Matula*..... Section *Matula*
   With one species.....8. *C. habgallae*
1. Basidiospores thin-walled, never observed in the context; imperfect receptacles unknown.....Section *Lomatina*
   2. Surface of receptacles smooth or with few hairs.....3
   2. Surface of receptacles with hyaline, simple to matted hairs.....4
3. Both large and small basidiospores present.....7. *C. simulans*
3. Basidiospores of only one size observed.....6. *C. pezizoidea*
   4. Spores large, 18–20 μ long, receptacles appearing like those of a small *Stereum*.....5. *C. stereoides*
   4. Spores rarely reaching 18 μ long.....5

5. Surface of receptacles pruinose, covered with short, interwoven hyphae..6
5. Surface of receptacles floccose to woolly.....7
6. Spores 14–18 × 4–5 μ.....1. *C. salicina*
6. Spores 10–11 × 5–6 μ.....2. *C. sarcoides*
7. Surface of receptacles floccose, spores 6–10 × 1.5–3 μ.....3. *C. flocculenta*
7. Surface of receptacles woolly, spores 6–7 × 2–3 μ.....4. *C. lanata*

Section 1. **Lomatina** (Karst.), W. B. Cooke, *sect. nov.*

Receptacles smooth, pruinose, floccose or woolly on the upper surface, with white to red or dark red hymenium, with chlamydospores embedded in the context in at least two species, without thick-walled basidiospores.

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Thelephora cruenta Alb. & Schw. Consp. Fung. 227. 1805.
Thelephora salicina Fries, Syst. Myc. 1: 442. 1821.
Corticium salicinum Fries, Epicrisis 558. 1838.
Corticium sanguineum Fr. *sensu* Karst. in Fungi Fenn. No. *132* (N. Y. B. G.). 1865; Not Bourd. & Galz. (= *Peniophora*).
Cytidia rutilans (Pers.) Quél. Fl. Myc. Fr. 25. 1888.
Lomatia salicina (Fr.) Karst. Bidr. Finl. Nat. Folk. **48**: 404. 1889.
Lomatina salicina (Fr.) Karst. Bidr. Finl. Nat. Folk. **48**: 404. 1889.
Cytidia cruenta (Pers.) Hert. in Rabenh. Krypt. Fl. Brand. **6**: 83. 1910.

Receptacles centrally or subcentrally attached at one point, otherwise free, 0.2–1 cm. in diameter, pezizoid, remaining single in spite of crowdedness, or becoming confluent, if confluent up to 5 cm. wide and individuals retaining identity by well marked areas of fusion, or losing their identity completely except for the points of attachment which remain evident; appearing resupinate or reflexed, margin inrolled on drying; 300–600 μ thick in section, hyphae of context mostly parallel with surface of receptacle, in the lower quarter to third curving outward to form the hymenium which is perpendicular to the surface of the pileus, hyphae closely compacted, with irregular gelatinous walls, with clamps; hymenium pinkish when faded, to dark red when fresh, appearing merulioid in some specimens, 40–50 μ thick; surface of receptacles appearing pruinose but composed of short bundles of parallel hyphae which may form zones in older specimens; sterile organs in the hymenium dendrophysoid, not readily found in most specimens examined, with finely branched or curved tips, filiform, brownish ("looped or crumbled" according to Burt); basidia 40–50 × 6–8 μ, 4-spored;

spores hyaline, smooth, apiculate, allantoid, 12–(14)–18 × 4–6 μ; spaces in hymenium filled with brownish, gelatinous, granular material.

Habitat: On wood and bark of trunks of deciduous trees including: *Salix* spp., *S. pentandra, S. aurita, S. capraea, S. cordata, S. discolor, S. fragilis, S. fluviatilis, S. grandifolia, S. lasiandra, S. longipes, S. nigra, S. purpurea, S. scouleriana, Alnus* spp., *A. incana, Populus* spp., *P. tremula, P. tremuloides, P. trichocarpa, Prunus serotina.*

Specimens have been examined from: **Africa: Algeria** (1); **Europe: Austria** (3); **Czechoslovakia** (1); **Finland** (4); **Germany** (1); **Italy** (2); **Latvia** (2); **Norway** (1); **Sweden** (2); **North America: Alaska** (1); **British Columbia** (5); **Manitoba** (2); **Nova Scotia** (1); **Ontario** (27); **Quebec** (8); **Alabama** (1); **Colorado** (5); **Connecticut** (4); **Idaho** (11); **Indiana** (1); **Louisiana** (1); **Maine** (2); **Massachusetts** (1); **Michigan** (13); **Minnesota** (2); **Montana** (1); **New Hampshire** (12); **New Mexico** (1); **New York** (23); **North Carolina** (1); **North Dakota** (1); **Ohio** (1); **Oregon** (1); **Pennsylvania** (4); **Vermont** (6); **Virginia** (2); **Washington** (3); **West Virginia** (1); **Wisconsin** (1); **Wyoming** (1).

At Iowa City, Iowa, G. W. Martin has found material roughly similar to this species on twigs of *Gleditsia triacanthos.* His specimens differ from typical *C. salicina* specimens by having smooth, pezizaform receptacles without hyaline matted surface hairs but with atrosclerotized "cortical" cells and by having yellow to brownish conducting organs apparently terminating certain undifferentiated hyaline hyphae in the context. The specialized cells thus formed measure 20–50 × 8–11 μ, appear like sunken gloeocystidia, are irregular in shape and were not seen in the hymenium but only in the trama. They were arranged regularly and lay in the general position of the direction of the hyphae, parallel with or becoming perpendicular to the substratum. Basidia and spores are similar in size and shape to those of *C. salicina* although the basidia tend to be swollen at the base. Until additional material is found this specimen is placed doubtfully with *C. salicina.* A specimen in the Ellis Collection, New York Botanical Garden, is labelled by Peck: *Exidia cinnabarina* Berk. & Curt.

2. **Cytidia sarcoides** (Fr.) *comb. nov.*

Thelephora sarcoides Fr. Elenchus 1185. 1828.
Corticium sarcoides (Fr.) Fr. Epicrisis 558. 1838.

Receptacles attached at one point, free elsewhere, back of receptacle gray, composed of bundles of context hyphae which are hyaline to pale yellow brown; hymenium flesh color to light purplish; basidia 50–60 × 6–8 μ, projecting from the hymenium at maturity, 4-spored; yellow conducting organs present, yellow chlamydospores 5–15 μ in diameter occur scattered throughout the context; context hyphae hyaline, with thickened gelatinous walls and clamps at the septa; hymenium compact, with yellow gloeocystidia 50 × 8–10 μ, and branched paraphyses 50× 3–5 μ; spores ovate, hyaline, smooth, apiculate, 10–11 × 5–6 μ.

Habitat: On bark of *Salix pentandra;* appearing like *C. salicina.*

Specimens examined: **Latvia:** Prov. Vidzeme, Vestiena. Dec. 26, 1933, K. Starcs *1373,* and Sept. 9, 1936, K. Starcs *4132.*

3. *Cytidia flocculenta* (Fr.), v. Höhn. & Litsch. In Oesterreichische Cortic. 61. 1907.

Thelephora flocculentum Fr. Elench. **1**: 184. 1828.
Corticium flocculentum Fr. Epicr. 647. 1838.
Cyphella ampla Lév. Ann. Sci. Nat. Bot. III. **9**: 126. 1848.
Auricularia leveillei Quél. Soc. Bot. 1879.
Auriculariopsis flocculenta (Fr.) R. Maire, Rech. Cyt. et Tax. 102. 1901.
Cyphella flocculenta (Fr.) Bres. Ann. Myc. **1**: 111. 1902.
Auriculariopsis ampla (Lév.) R. Maire, Soc. Myc. Fr. Bull. **18** Suppl.: 102. 1902.
Stereum pubescens Burt, Mo. Bot. Gard. Ann. **7**: 178. 1920.

Receptacles 6–10 mm. in diameter, confluent up to 2–3 cm. long, attached at and near a central point, free over remainder of upper surfaces; free surface covered with a coarse tomentum, gray to tan in color, zonate, tomentum made up of hyaline hyphae which are united in bundles, the hyphae branch and may connect the bundles, the bundles branch and anastomose, bundles loosely arranged, hyphae parallel, with thick, gelatinous walls, septa not evident, hyphae 5–8 μ in diameter, bundles 25–50 μ in diameter, with numerous hyphae in each, bundles and hyphae less orderly, more interwoven, toward the receptacle surface; surface of receptacle marked by a

dark zone from which the tomentum appears to arise; hyphae of receptacle densely interwoven, more or less parallel, becoming perpendicular to the receptacle in the subhymenial region, no sterile elements seen, pileus 500–600 μ thick in section, hymenial layer smooth, reddish to dull purple, 75 μ thick, darker in color than the context in sections; basidia 20–30 × 4–5 μ, 4-sterigmate; densely compacted into a layer, without cementation, arising from a brownish subhymenial area, no conducting organs evident; small clamps present at the base of basidia and in the subhymenial area; context hyphae elongate, with gelatinous walls and large clamps at which the hyphae sometimes branch, 4 μ in diameter; spores hyaline, smooth, apiculate, allantoid, 6–10 × 1.5–3 μ.

Habitat: On bark of deciduous trees and shrubs including species of *Alnus, Salix, Betula, Prunus* and *Populus; Salix alba, Populus nigra* and *P. pyramidalis.*

Specimens have been examined from: **Europe: Austria** (1); **France** (1); **Germany** (2); **Hungary** (1); **Jugoslavia** (1); **Russia** (2); **North America: Ontario** (1); **Montana** (1); **Wyoming** (1); **Australia: South Australia** (1).

4. **Cytidia lanata** sp. nov.

Receptaculum cupuliforme, centro affixum, coriaceo-gelatinosum, sparse, extus lanatum, dilute brunneum, 1–3 cm. latum; basidiis clavatis, 4-sporis, 23–28 × 4–6 μ; sporis hyalinis, oblongo-cylindraceis, 6–7 × 2–3 μ. Hab. in cortice *Betulae* spec. TYPUS: **Idaho:** Moscow Mt., Latah Co., H. C. Aase, May 14, 1933. (WSC. *170986.*)

Receptacles 1–3 cm. in diameter, scattered on the branch, attached at and near a central point, free over remainder of upper surface; free surface covered with a tan to brownish woolly tomentum, more or less zonate, made up of hyaline hyphae which are united into bundles which are more compactly arranged toward the outside; the bundles merge and anastomose and are connected by individual hyphae, hyphae 5–8 μ in diameter, bundles 25–50 μ in diameter; hyphae of tomentum originate from the context through a zone of darkened tissue; hyphae of context densely interwoven, more or less parallel, without clamps at the infrequent septa, with gelatinous walls, becoming perpendicular to the receptacle in the subhymenial region of each annual increment, no sterile elements seen, receptacle 600–800 μ thick in section; hymenium smooth to

somewhat wrinkled, dull purple in color, in section brownish, 50–60 μ thick, in two to three layers, the lower layers of which appear as brown palisades of collapsed cells through which hyphae penetrate to form a context from which an additional hymenial layer arises; no sterile elements seen in the context nor in the hymenium; basidia 23–28 × 4–6 μ, with 4 sterigmata, with small clamps at their bases; spores hyaline, smooth, apiculate, ovate to oblong-ellipsoid, flattened on one side, 6–7 × 2–3 μ.

Habitat: Bark of *Betula* sp.

Specimen examined: **Idaho**: Moscow Mt., Latah Co., May 14, 1933, H. C. Aase (WSC *170986*), TYPE.

5. **Cytidia stereoides** sp. nov.

Receptaculum coriaceo-gelatinosum, centro affixum, confluens vel persistens cupulare, extus pallide hirsutum, 0.2–1 cm. latum; hymenio leviusculo, nudo, pallide carneo-purpurascente; basidiis 40–55 × 7.2–14.4 μ, sporis hyalinis, continuis, cylindraceis vel allantoideis, 18–20 × 7–7.5 μ.

Receptacles attached at a more or less central point, free for 1–3 mm. around the margin, 0.2–1 cm. in diameter, becoming confluent in patches up to 2 cm. wide, 400–500 μ thick in section, tissue mostly of hyaline hyphae, which are long, slender, gelatinous walled, densely interwoven, 3.5–4.5 μ in diameter, with clamps, becoming more or less parallel below the hymenium; upper surface of pileus composed of hyphae extending upward from the surface in parallel arrangement, of varying length, smooth, hyaline, gelatinous walled, rarely septate, forming a palisadoderm or a trichoderm but may become agglutinated into bundles; hymenium pinkish flesh-color, becoming pinkish drab on drying, with slender dendrophyses branching at clamp connections, 40–144 × 5–11 μ; basidia 40–55 × 7.2–14.4 μ, extending beyond the hymenium at maturity, with 4 sterigmata up to 7 μ long; spores hyaline, smooth, apiculate, cylindric to allantoid, 18–22 × 7–7.5 μ.

Habitat: On dead branches of shrubs including *Ceanothus velutinus*, *Cercocarpus ledifolius* and *Purshia tridentata*, appearing like a small *Stereum*.

Specimens examined: **California**: Siskiyou Co.: North base of Mount Shasta, 4000–5000 feet: April 7, 1947, W. B. & V. G. Cooke *19329*, TYPE; April 9, 1947, W. B. & V. G. Cooke *19411*. Both in Herb. W. B. C.

6. *Cytidia pezizoidea* (Pat.) Pat. Tax. Hymen. 54. 1900.

Corticium pezizoideum Pat. Jour. de Bot. **5**: 314. 1891.
Cytidia wettsteinii Bres. Denk. k. Akad. Wiss. Wien **83**: Extr. 6. 1907.
Cytidia tremellosa Lloyd, Myc. Writ. **4**: 516, *f. 512, 513.* 1912.
Corticium wettsteinii (Bres.) Sacc. & Trott. Syll. Fung. **21**: 400. 1912.

Receptacles gregarious, 1–5 mm. in diameter, attached at one point, free around the edges, surface smooth or with few hairs; when confluent appearing merulioid, edges white, hymenium drab; dendrophyses strongly developed, basidia 4-spored, clamped at the base, hyphae of trama with strongly developed clamps, with gelatinous walls; spores hyaline to faintly colored, smooth, ovate, apiculate, 8–10 × 5–6.5 μ.

Habitat: On rotting wood.

Specimens have been examined from: **Asia: Malaya** (1); **North America: Louisiana** (2); **Panama** (1).

Cytidia wettsteinii Bres. is placed in synonymy with *C. pezizoidea* (Pat.) Pat. on the basis of the description in Saccardo (l.c.). The spores are listed as 9–11 × 6–8 μ and the other characters described are not sufficiently distinct to warrant its retention as a separate species.

7. *Cytidia simulans* Lloyd, Myc. Writ. **6**: 991, *pl. 159, f. 1772.* 1920.

Receptacles discoid, concave, attached at a central area, 1–1.5 cm. in diameter, fleshy gelatinous, hymenial surface somewhat wrinkled, pale brown; upper surface white, smooth or with a few hyaline hyphae giving a white pruinose aspect to it; clamp-connections well developed on the gelatinous walled context which are arranged parallel with the receptacle surface but are more or less interwoven; hymenium arising from the context hyphae which become perpendicular to the context just below the subhymenial region; two or three palisades of hymenium-like tissue indicate that the receptacles are probably perennial; no sterile elements seen in the hymenium nor the context tissues; only one type of basidium seen, 32–35 × 3.5–4.5 μ, but some have two sterigmata, others four; spores of two sizes, those produced on the 4-sterigmate basidia are hyaline, smooth, thin-walled, apiculate, allantoid, 6.3–7.5 × 2–2.5 μ

(Lloyd's conidia), those produced on the 2-sterigmate basidia ovate-cylindric to suballantoid, hyaline, smooth, thin-walled, apiculate, 8.5–12.5 × 5.8–6.5 μ.

Habitat: On dead wood.

Type specimen was examined from the **Union of South Africa.**

Section 2. **Matula** (Massee) sect. nov.

Receptacles pruinose or floccose on the upper surface, with whitish to flesh colored hymenium, with thick-walled basidiospores embedded in the context.

8. *Cytidia habgallae* (Berk. & Br.) G. W. Martin, Lloydia **5**: 160. 1942.

Corticium habgallae Berk. & Br. Jour. Linn. Soc. Bot. **14**: 72. 1873.
Peniophora habgallae (Berk. & Br.) Cooke, Grev. **8**: 20. 1879.
Matula poroniaeformis (Berk. & Br.) Massee, E. Micr. Sc. Jour. **4**: 173. 1888.
Michenera rompelii Rick, Ann. Mycol. **2**: 243. 1904.
Matula rompelii (Rick) Lloyd, Myc. Writ. **2**: 391. 1908.
Cytidia cornea Lloyd, Myc. Writ. **5**: 646. 1917.
Aleurodiscus corneus Lloyd, Myc. Writ. **6**: 930. 1920.
Aleurodiscus capensis Lloyd, Myc. Writ. **6**: 930. 1920.
Glocosoma capensis Lloyd, Myc. Writ. **6**: 1088. 1921.
Aleurocystus capensis Lloyd (as McGinty), Myc. Writ. **6**: 1088. 1921.

Fructifications single to gregarious, irregularly discoid, attached at the center to substipitate, free at the margin, becoming reflexed, when dry cream to yellow or vinaceous, becoming brown, when fresh reported by Martin as at first white, then pinkish buff (R), pinkish cinnamon (R), Sayal Brown (R), vinaceous buff (R), to fawn color (R); in section up to 1–1.2 mm. thick, reported by Petch as 3 mm. thick, but that probably at center including attachment; surface hyphae not differentiated, usually agglutinated into bundles; tissue next to surface and subhymenial areas of densely interwoven gelatinous hyphae, inner context hyphae not as densely interwoven, mostly irregularly parallel with the substratum, with well developed clamps at each septum.

In non-basidial fructifications spores are produced in chambers in discoid receptacles, intercalary at the distal side of clamp-connections; the contents of a cell which will produce a spore are

rounded up and a gelatinous wall is developed around them; the lumen is smooth or projects conically into the wall at 6–8 places of which three may be visible in one plane: these probably represent germ pores; spores globose to ovoid, 18–23 μ in diameter, or 15–18 × 20–28 μ, wall of two layers, the outer highly refractive, 0.6–0.8 μ thick, the inner, which may be concentrically zoned, 3–4 μ thick.

In basidial fructifications the hymenium is composed of three elements: cystidia hyaline to yellowish, with smooth to roughened walls, fusiform, seta-like, embedded in the hymenium or projecting as much as 35–45 μ, 60–100 × 13–25 μ; paraphyses somewhat dichotomously branched, 60–70 × 2–3 μ; basidia 4-spored, hyaline, 60–70–(100) × 15–25 μ, clavate, with slender stalk and swollen tips, sterigmata up to 10 μ long; spores at first thin-walled, smooth, hyaline, apiculate, ovate to ellipsoid, reaching 18–25 × 10.8–18 μ, finally thick-walled, smooth, yellowish, ovate to ellipsoid, with two germ pores of which one is near the apiculum, the other opposite it, 16–22 × 12–16 μ, liberated from the hymenium as in other Basidiomycetes whose spores are violently discharged but some becoming embedded in the tissues of the receptacles.

Habitat: On twigs, branches and dead wood.

Specimens have been examined from: **Australasia: Tasmania** (1); **Africa: Union of South Africa** (1); **Central America: Panama** (2); **South America: Colombia** (1).

The writer wishes to take the opportunity to thank the curators of the following herbaria for the opportunity afforded him to study the specimens under their care: Mycological Collections and Forest Pathology Collections, Bureau of Plant Industry; C. G. Lloyd Mycological Collections, New York Botanical Garden, Missouri Botanical Garden, University of Michigan, Oberlin College, Pennsylvania State College, L. O. Overholts Herbarium, University of California, Herbarium of W. H. Snell, University of Minnesota, University of Idaho Forest Pathology Herbarium, University of Toronto, Science Service Herbarium, Ottawa, University of Manitoba, State College of Washington, State University of Iowa. Previously published descriptions and notes and unpublished notes by various collectors and students, as well as the writer's observations,

have been drawn upon in the preparation of the above descriptions. Donald P. Rogers has checked the latin diagnoses. Drs. D. P. Rogers, G. W. Martin and H. S. Jackson have read the manuscript and offered valuable suggestions.

Type specimens of *Cytidia lanata* and *C. stercoides* have been deposited in the herbarium of the Department of Plant Pathology of the State College of Washington.

WASHINGTON STATE COLLEGE,
PULLMAN, WASHINGTON

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ARACHNOPEZIZA OBTUSIPILA GRELET DESCR. EMEND.

RICHARD P. KORF [1]

Arachnopeziza obtusipila Grelet was described in a rather obscure journal, and has apparently never been recorded in the standard abstracting publications. In the original description, Grelet indicated a single collection, which must be the type. The type specimen is now nearly useless, as it bears only a few sterile fragments of apothecia. There are several other collections of this species in his herbarium, from which the species must now be interpreted. Of these, one is marked "forme minor," which is the type specimen of *A. obtusipila* var. *minor* Grelet; it, also, is in a poor state of preservation. The varietal name is to be published, posthumously, in the near future.

There is but one collection in the Grelet herbarium which is well preserved, and fortunately the material is fairly abundant. This is the specimen collected by A. de Crozals, XII. 1927. It exhibits certain striking differences when compared with the published description and figures: the measurements throughout are distinctly smaller than those given by Grelet. In fact, they correspond rather neatly to the manuscript variety *minor*.

It is the writer's belief that the evidence at hand indicates variety *minor* to be a direct synonym of *A. obtusipila*, and that the measurements given for the latter were recorded incorrectly, presumably because of an error in the microscope calibration. The writer postulates that the type specimen of *A. obtusipila* was already beyond use when the species was again encountered by Grelet. It is assumed that he drew up the description of variety *minor*, using a corrected microscope calibration, by comparison with his published account rather than with the type specimen.

In the same paper in which *A. obtusipila* was described, Grelet erected three other new species: *Belonidium albo-rubrum, Lach-*

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TABLE I

LACHNELLA GRISEA GRELET

	Grelet	Korf	Grelet × K*
Hair width	4–5 μ	2.7–3.4(4.1) μ	3–3.75 μ
Ascus width	5–7 μ	4.8–6.1 μ	3.75–5.25 μ
Ascus length	65–90 μ	51–63(68) μ	48.75–67.5 μ
Spore width	2.5–3 μ	2–2.7 μ	1.9–2.25 μ
Spore length	8–17 μ	9.6–15 μ	6–12.75 μ
Paraphysis width	2.5–4 μ	2–2.7 μ	1.9–3 μ

* K = 0.75.

nella grisea, and *Mollisia lilacea*. Of these, the writer examined material of only *L. grisea* while he was at Paris. A parallel summary (TABLE I) is given of the dimensions of certain microscopic features (1) as described by Grelet, (2) as determined by the writer from an examination of the type specimen (11. X. 1916), and (3) of Grelet's original measurements multiplied by the assumed error factor in calibration (K = 0.75). It will be noted that the last two columns agree rather well, and that the correct interpretation of *L. grisea* requires appreciably smaller microscopic measurements than those originally given.

Now let us examine the case of the one well-preserved specimen of Grelet's *A. obtusipila*. In all likelihood, the original description was drawn up using the same microscope calibration as was used for *Lachnella grisea*. A parallel summary (TABLE II) is given of the dimensions (1) in Grelet's original description, (2) as determined by the writer from examination of the authentic specimen, (3) as determined by Dr. Marcelle Le Gal (*pers. comm.*) from the

TABLE II

ARACHNOPEZIZA OBTUSIPILA GRELET

	Grelet	Korf	Le Gal	Minor	Grelet × K*
Apothecial diameter	200–400 μ	225–300 μ	—	200–325 μ	150–300 μ
Hair width	5–6 μ	3.4–4.8 μ	—	—	3.75–4.5 μ
Hair length	50–125 μ	(40)70–100(120) μ	—	—	37.5–93.75 μ
Ascus width	10–12 μ	7.5–10.2 μ	8–9.5 μ	—	7.5–9 μ
Ascus length	75–85 μ	62–73 μ	70–86.4 μ	—	56.25–63.75 μ
Spore width	4–5 μ	2.7–3.4 μ	2.75–3.5 μ	3–4 μ	3–3.75 μ
Spore length	25–35 μ	16.4–26(30) μ	17.5–27.5 μ	18–32 μ	18.75–26.25 μ
Paraphysis width	2–2.5 μ	1.4 μ	1–1.5 μ	1–2 μ	1.5–1.9 μ

* K = 0.75.

same specimen, (4) in Grelet's unpublished description of variety *minor*, and (5) of Grelet's original description of *A. obtusipila* multiplied by the assumed error factor. The similarity in the measurements of the last four columns is rather striking.

The writer, in a letter to Dr. Le Gal, suggested the hypothesis that Grelet's measurements were too large. She, however, is not convinced that this interpretation is correct. An examination of the spores and asci (but apparently not the sterile elements) did not allow her to reach the same conclusion in regard to *Belonidium albo-rubrum;* in fact, she finds the published drawings were apparently made from too young material, and that the spores figured are too short. Unfortunately, she was unable to locate material of *Mollisia lilacea* in Grelet's herbarium for an investigation of that species.

On the basis of the data presented in the two tables, the following emended description of Grelet's species is provided.

ARACHNOPEZIZA OBTUSIPILA Grelet, L'Amateur de Champignons 8[3]: 45. 1922, *descr. emend.*

SYNONYM: *Arachnopeziza obtusipila* Grelet var. *minor* Grelet, *mss.* (fasc. 22, Les Discomycètes de France d'après la classification de Boudier, to be published in the Revue de Mycologie).

Subiculum whitish, scanty; hyphae 2.7–4.8 μ wide, fairly thick-walled, smooth or slightly roughened; septa occasional. **Apothecia** gregarious, sessile, 225–300 μ in diameter, drying turbinate; *receptacle* white, bearing hairs over the surface, tan on drying; *margin* acute to obtuse, usually elevated on drying; *disc* white, plane to concave on drying. **In section:** *hymenium* ca. 75 μ thick; *hypothecium* not differentiated; *medullary excipulum* none, or forming a layer $< 25\ \mu$ thick, of thin-walled, long-celled, intertwined hyphae; hyphae ca. 1.4 μ wide, hyaline; *ectal excipulum* of one to a few layers of thick-walled, gelatinized, short-celled, parallel hyphae; hyphae 5.5–8.2 μ wide, hyaline, giving rise to hairs. **Hairs** hyaline, 4.1–4.8 μ wide below, tapering gradually to an apex 3.4–4.1 μ wide, the apex occasionally slightly swollen; medium thick-walled, (40)70–100(120) μ long, at times constricted at the septa, smooth or with external particles. **Asci** 8-spored, arising

from croziers, clavate; the tip rather abruptly truncate, with the pore blueing in iodine; 62–73 × 7.5–10.2 μ. **Ascospores** hyaline, subfusoid, attenuate below, irregularly biseriate, 3(4)-septate at maturity, 16.4–26(30) × 2.7–3.4 μ. **Paraphyses** hyaline, simple or branched, filiform, apex often variously misshapen, about as long as the asci, ca. 1.4 μ wide; septa few.

The writer is particularly indebted to Professor Roger Heim and to Dr. Marcelle Le Gal for their permission to examine specimens in the Muséum National d'Histoire Naturelle, for permission to examine the Grelet manuscript, and for the many courtesies extended to the writer during his stay at that institution. Professor W. Lawrence White, of Harvard University, has most kindly provided the author with certain literature not otherwise available to him.

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THAXTEROGASTER—A NEW LINK BETWEEN GASTROMYCETES AND AGARICALES

R. SINGER

(WITH 2 FIGURES)

In the course of a study of the Secotia preserved at the Farlow Herbarium, I noticed a specimen with large rusty colored verrucose spores deposited there under the name of *Secotium magellanicum* Thaxter. Three years later, traveling for the Instituto M. Lillo, Universidad Nacional de Tucumán, Argentina, to Tierra del Fuego, I found two species congeneric with Thaxter's fungus and both obviously belonging to the Secotiaceae. I was unable to find Thaxter's herbarium name published anywhere in the literature and assume that it has never been published. Nevertheless, I am not quite sure which of the two species collected by me is identical with Thaxter's species. I assume it is the one described here as *Thaxterogaster magellanicum* gen. nov. spec. nov., but it is here considered a new species in order to avoid later difficulties in case discrepancies between the characters of my *T. magellanicum* and Thaxter's *Secotium magellanicum* ined. (?) should show up. Nevertheless, there can be no question about the fact that Thaxter was the first to collect any American representative of this new genus which, therefore, is named for him. It is possible that some of the rough spored species of *Secotium* described from other parts of the world, especially New Zealand and Australia, belong here, but since I have not studied them, I do not believe they should be included on the basis of their descriptions only. *S. Gueinzii* Kunze, the type species of the genus *Secotium*, does not seem to belong here since Corda (**1854**) who had original material from Kunze indicates and draws the spores smooth (*"glabris," "sehr stark vergrössert"*) and says that they are slightly yellowish white, while those of *Thaxterogaster* are much deeper colored.

The species determined as *S. Gueinzii* by Cunningham from Tasmania and mentioned by Fischer (**1933**) as having rough or warty spores is probably a different species.

Thaxterogaster gen. nov. Carpophoris subhypogaeis, stipitatis, angiocarpicis vel subhemiangiocarpicis, unipileatis; gleba e loculis minutis irregularibus consistente, *Hymenogastrorum* modo albovenosa, ferrugineo-ochracea, haud pulveracea, hymeniifera; columella praesente sed interdum apicem peridii ramificationibus tenuissimis tantum attingente, cum stipite constanter confluente; stipite bene evoluto et cum peridio in juventute semper, senectute saepius contiguo; cortina et velo strato externo peridii homologis semper praesentibus et saepius distinctissimis; cavitate annuliformi magna inter columellam et peridium gleba impleta; columella stipiteque solidis cum peridio carnosis; pileo (peridio) globoso, semiglobato-pileiformi, conico, vel cylindrico; cystidiis nullis; pseudoparaphysibus plerumque praesentibus; basidiis tetrasporis; sporis symmetricis vel paucis subasymmetricis acrogenis, maiusculis, brunneis vel ferrugineis, Cortinariorum modo exosporio verrucoso praeditis; sterigmatibus bene evolutis, apicalibus; tramate ex hyphis elongatis fibulatis hyalinis constante. Rhizomorphis albis frequentibus.—Species typica: *Thaxterogaster magellanicum* Sing. sp. n.

Thaxterogaster violaceum Sing. spec. nov. Characteribus generis. Peridio violaceo-caeruleo, demum pallescente, subgloboso; columella apicem peridii haud constanter attingente; pseudoparaphysibus sparsis; basidiis 40–52 × 10.8–12.5 μ; sporis 15–17.8 × 9.5–9.7 μ. Odore nullo.—In humo in silvis nothofagineis montanis (*Nothofagus pumilio*), gregatim. Argentina: Lago Fagnano (Kamel), Tierra del Fuego prope ripam meridionalem partis orientalis lacus versus latus montis "Observación." Typus a R. Singer collectus (*M 362*, LIL, 19–II–1950).

The buttons consist of two usually almost equal portions, the upper representing the pileus, the lower the stipe, both subglobose and slightly constricted at the junction. In this stage, until maturity, all parts are more or less violet blue (near "mauvette" Maerz & Paul), but after maturity is reached they bleach to pl. 42, 2-B (Maerz & Paul) or paler; only occasionally a few brown spots may be present. No rupture between the lower margin of the peridium and the stipe takes place at maturity, nor does the peridium become longitudinally split. The mature peridium shows a poorly differentiated outer layer which is sericeous-arachnoid-fibrillose but completely smooth, distinctly fibrillose near the point of attachment to the apex of the stipe where it forms a more or less distinct belt-shaped veil which is somewhat lighter colored than the rest of the surface of the carpophores and becomes whitish sooner than the latter; the inner portion of this veil becomes an

arachnoid cortina as in the Cortinarii as soon as the expansion of the carpophore progresses more rapidly than the growth of the hyphae of the veil; the diameter of the sterile cortex of the pileus-portion (the peridium proper) is about 1 mm. excepting the apical portion where it joins with the columella or its branches and reaches up to 4 mm. in diameter; the entire pileus-portion of the carpophore is 19–36 mm. broad at maturity. The columella is as broad as the stipe but tapers strongly upwards to a diameter of about 1 mm.; more rarely the columella does not reach the apex

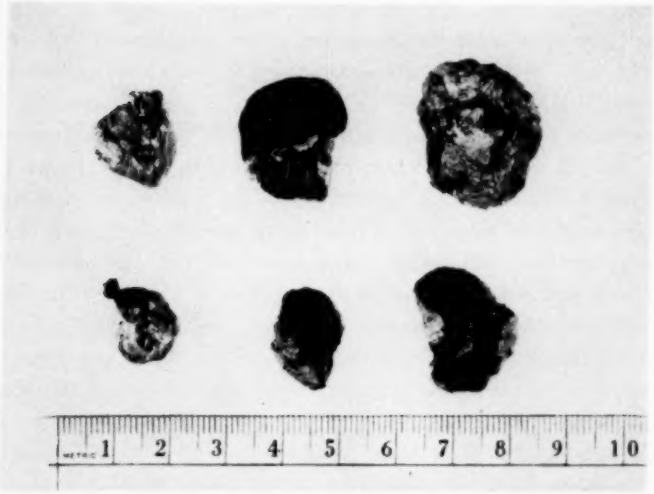

FIG. 1. *Thaxterogaster violaceum* Sing. Carpophores whole and in section. Photo. Singer and Brennan.

of the peridium but ramifies into very thin tramal plates at a short distance from the apical portion of the peridium. The stipe is concolorous with the peridium and soon becomes relatively short, i.e., does not stretch at the same ratio as the pileus grows; it is solid as the columella, 5–17 × 3–16 mm., versiform, slightly sericeous; all surfaces, those of the stipe as well as those of the pileus, are completely dry, neither viscid nor hygrophanous. The gleba fills the entire "ring-hole" between the columella as central axis and the sterile rind of the peridium; it consists of irregularly ar-

ranged chambers formed by concolorous tramal plates which are beset with a hymenium of basidia which, at an early stage, begin to discharge spores wherefrom the interior of each chamber becomes rusty-ocher-brown; the consistency of all tramal tissues of the carpophore is fleshy, never tough, and the color of the context is similar to that of the outer surfaces, or slightly paler; the entire height of the carpophores reaches 17–33 mm. but only the upper half of the peridium shows above the ground; there is no odor whatever, even in overmature specimens.—Spores melleous and smooth when immature, most of them definitely symmetrically attached, only a few, originating from sterigmata which are slightly below the apex of the basidium and more sickle-shaped than ordinarily may be termed "subasymmetrical"; when mature they are distinctly thick-walled, the wall consisting of three layers, (1) an innermost hyaline or subhyaline endosporium, further outwards the rusty colored episporium which is smooth but beset with the deeper colored warts constituting the exosporial ornamentation which shows no plage; there is no germ pore and both ends of the spores are rounded; their shape is ellipsoid to ellipsoid-oblong, without suprahilar depression or applanation, 15–17.8 × 9.5–9.7 μ. Basidia clavate, but in some individuals slightly attenuate at the apex or with a very slight constriction just below the apex (as sometimes seen in *Gymnopilus*), with most of the sterigmata strictly apical and very straight and upright as in *Galeropsis*, a few attached lower and then more sickle-shaped, all hyaline and rather voluminous, 40–52 × 10.8–12.5 μ. Cystidia none. A few pseudoparaphyses are usually present. Trama of the tramal plates consisting of rather crowded subparallel hyphae with clamp-connections; hyphal walls all smooth, slightly thickened in some hyphae of the peridial layer, otherwise rather thin-walled. The carpophores are found in deep soft humus in shady high woods of the mountain region, consisting mostly of *Nothofagus pumilio*, with very few evergreen *Nothofagus* intermixed but not close to the habitat, the youngest stages developing subhypogeously, fruiting in February (but probably also before and afterwards). Near the western end of Lago Fagnano (Kamel), between the south shore of the lake and the wooded mountain side of Cerro Observación, Tierra del Fuego, Argentina.

Thaxterogaster magellanicum Sing. spec. nov. A species altera differt peridio numquam violaceo-caeruleo, magis versiformi, circum apicem stipitis demum aperto in plurimis individuis vel in parte superiore fracta *Secotii agaricoidei* modo (fissionibus longitudinalibus), brunnescente, sublubricante, columella haud semper apicem versus attenuata quamquam in plurimis speciminibus distincte attenuata sit, numquam ramificata sed semper apicem peridii attingente; pseudoparaphysibus numerosis conspicuisque; basidiis 34–36 × 7.5–8 μ; sporis 13–17 × 8.8–12 μ; odore maturitate distincto, aromatico, iucundo.—In humo in silvis nothofagineis montanis et planitiei (*Nothofagus pumilio* et *N. antarctica*), gregatim. Argentina: Lago Fagnano (Kamel) et in regione silvestri Rio Grande, prope Estancia "Nueva Argentina." Typus a R. Singer collectus (*M 515*, LIL. 10–II–1950); paratypi *M 178, M 205, M 259, M 296, M 299.* Idem fungus prope Ushuaia (litus Beagle Canal) observatus est ab A. Digilio.

This species is much like the preceding one. It differs in the absence of violet-blue tones, and in a number of less sharp or less striking characters such as the less constantly subglobose shape of the peridium (pileus) which may be conical to cylindrical, rarely ovoid or ellipsoid, but often hat-shaped as in many agarics, especially in those specimens where, in contrast to what is observed in

FIG. 2. *Thaxterogaster magellanicum* Sing. Carpophores whole and in section. Photo. Singer and Brennan.

T. violaceum, the peridium is dehiscent from the apex of the stipe exposing the gleba. Other distinguishing characters are the less constantly attenuate columella which, however, constantly reaches the apex of the peridium; the usually more frequent and numerous pseudoparaphyses, the slightly narrower spores with perhaps a larger number of subasymmetrical spores in an average preparation; the shorter basidia; the presence of a characteristic pleasant odor in mature and over-mature specimens, attracting insects which frequently make openings in the mature peridium (not so in the inodorous stage) so much so that it is difficult to find an odoriferous specimen without such holes; the slight lubricosity found in a large number of specimens; and finally its occurrence under not only *Nothofagus pumilio* but *N. antarctica* with an extension of its area northwards into the region of the rolling hills and flatlands of Rio Grande, far away from the Cordillera. Otherwise the characters and appearance of this species are identical with that of *T. violaceum,* its size being the same or perhaps slightly larger in some specimens (reaching 40–80 mm. in height) except for the stipe which is somewhat longer as compared with the pileus rather than of about equal length and volume as seen in the buttons of *T. violaceum* or shorter than the pileus portion as seen in mature specimens of the latter species.

When studying the specimens of *Thaxterogaster* in nature, the characters and position of *Thaxterogaster* become more obvious than in a herbarium study. In the first place, observations in the field show the species of *Thaxterogaster* to be typical forest-inhabiting humicolous species with decidedly fleshy carpophores, not as Fischer (**1933**) characterizes them, "derb" and not growing in mesophytic or xerophytic conditions. Furthermore the observations in the field make it quite certain that both species are not aberrations of certain agarics such as the gastromycetoid forms or conditions of *Boletinus decipiens.* This is shown by the absence of any forms with constantly expanding pileus and geotropically arranged hymenophore, the definitely angiocarpous, or more rarely almost angiocarpous development of the carpophores, and the constant correlation of the gastromycetoid characters in all specimens of the groups and in all collections made at different localities. It

is also worth mentioning that the aborted forms of agarics with gastroid tendencies (such as the *Lentodium*-form of *Panus tigrinus* (Bull. ex Fr.) Sing. with non-geotropic hymenophore, or the gastroid condition of *Boletinus decipiens*) have either no hymenium at all, or are provided with the same type of basidia as found in the normal form, viz. basidia forming the spores on sterigmata not continuing the longitudinal axis of the spores; consequently the spores are all of the asymmetrical type of the Agaricales. In *Thaxterogaster,* the longitudinal axis of the spores continues the direction of the sterigmata in most cases; only rarely, the more sickle-shaped sterigmata that are somewhat removed from their ordinary strictly acrogenous position form slightly asymmetrical spores which are a minority among the spores. The hymenophores of *Thaxterogaster* are chambers as often observed in the Hymenogastraceae, but the tramal surfaces are covered with a typical hymenium which, even if the peridium is splitting or dehiscent at the apex of the stipe, forms mature spores in large number long before such an opening has taken place. All these facts lead to the conclusion that *Thaxterogaster* is a genus different from *Secotium* not merely because of the warty spores but on the base of several other characters of taxonomic importance, yet, still a Gastromycete, at least in the classical sense (*i.e.* unless all Podaxales are excluded). They are definitely not aborted agarics, nor are they congeneric with such Secotia as *Secotium agaricoides* (Czern.) Hollós or *S. arizonicum* Shear & Griff.

While it is true that the spores of the herbarium specimens immediately suggest *Cortinarius* spores, the affinity between *Thaxterogaster* and *Cortinarius* becomes even more evident when both genera are studied in their natural habitats in the woods of Tierra del Fuego. Both species when first collected, and even in subsequent collections, are invariably at first glance identified as young Cortinarii. They are deceptively similar in every respect except for the configuration of the hymenophores (as is the case in *Cantharellus cibarius* and *C. lateritius,* or in *Pseudofavolus* and *Mycobonia* spp.), the development of the carpophores, and the axial symmetry of the spores. The first of these three characters can be seen only if the specimen is cut in half; the second and third

can be noticed only by careful study of successive stages and spore preparations under the microscope. The sublubricous surface of *Thaxterogaster magellanicum* and the violet-blue pigment of *T. violaceum* are additional reasons leading to a mental association between these Gastromycetes and the Cortinarii. Especially the violet-blue pigment is extremely suggestive of the Cortinariaceae (*Inocybe* and *Cortinarius*), especially some species of the local flora before the pilei expand. The cortina occurring in both species is in appearance and structure a typical *Cortinarius*-cortina, and the outermost layer of the veil may be compared with the universal veil occurring in those Cortinarii with the belt-like rings (subgenus *Telamonia*). Furthermore, it is remarkable that in a basidiomyceteous flora comparatively poor in species and genera such as the mycoflora of the wooded areas of Rio Grande and the Argentine part of Lago Fagnano (Kamel), Tierra del Fuego, the genus *Cortinarius* is represented by a considerable number of species dominating the agaric flora of the whole territory. As if this were not enough, the observer notices that here, more than in any other area known to me, a strikingly high percentage of the individual fruiting bodies of Cortinarii shows abnormally formed hymenophores such as lamellae with strong oblique anastomoses, with rudimentary chambers of large diameter etc. These aberrations are most common among species with either violet or viscid pilei, and are always accompanied by specimens with normal lamellae at least in a neighboring population in the same general locality. A microscopical check showed that, in contrast to *Thaxterogaster*, in the gastroid aberrations of *Cortinarius* (often accompanied by slow expansion of the pileus), the spores are typically asymmetrical as in ordinary forms of *Cortinarius* all over the world.[1] If this evidence is added to the facts shown in the detailed description of the genus and the two species, one cannot doubt the affinity—in a strictly phylogenetic sense—of *Thaxterogaster* and *Cortinarius*. The structure of the spore wall, the shape of the spores (except for

[1] The detailed study of the Cortinarii of Tierra del Fuego which Dr. Alexander H. Smith has kindly consented to undertake on the basis of my collections, has not been finished at present; therefore, no determinations of the species involved here are indicated in the present paper, but will be supplied later.

axial symmetry) and the color, the shape of the basidia, the number of sterigmata, the presence of clamp connections, the regular hymenophoral trama, the absence of cystidia, the structure and appearance of the cortina, the origin of the veil, the habitat in the humus under Fagales, all this is identical in *Thaxterogaster* and *Cortinarius*.

The genus *Thaxterogaster* fills in another gap between the Agaricales and the Gastromycetes. The bridges between the two forms of Higher Basidiomycetes (the "astrogastraceous" bridge, the links between *Galeropsis* and *Cyttarophyllum*, between *Montagnea* and Coprinaceae, between *Truncocolumella* and the Boletaceae being the ones that have been established until now, with several possible links like *Richoniella*, *Battarraea*, *Torrendia* still to be investigated) are now supplemented by a direct connection between *Thaxterogaster* (Secotiaceae) and *Cortinarius* (Cortinariaceae). The affinity of gastromycetous genera with certain groups of agarics and boletes has now entered the modern text books on mycology, and can hardly be denied by any serious mycologist. Gäumann (**1949**) says "Manche Agaricales zeigen denn auch engere Beziehungen zu den einfachern Gastromyceten als zu den Aphyllophorales und müssen vielleicht, im Zusammenhang mit den hier nicht besprochenen Secotiaceen und Podaxaceen, von diesen abgeleitet werden." Similar conclusions are drawn by Langeron (**1945**).

It is quite different as far as the *direction* of the evolution is concerned. In my forthcoming book "The Agaricales" (to be published by the Instituto M. Lillo, Tucumán, Argentina in June 1950), I have attempted to discuss the question of Agaricales phylogeny without emphasizing my own theory because neither theory can be considered as being a valid scientific fact. However, an attempt to make it appear as if the gastromycete forms close to the agarics were *established* or *proved* to be degraded descendants of the Agaricales, and the opposed theory as having been *refuted*, is likely to cause confusion and misunderstandings. Heim (**1948**), in a survey on phylogeny and natural classification of the Macro-Fungi, has made an interesting contribution in the course of his discussion of this interesting subject, and our French col-

league and friend may be assured that the arguments in favor of his own views will be appreciated in any serious effort to weigh the probabilities; for, in discussing the derivation of the Agaricales and Gastromycetes, at least at the present moment, we can do no more than weigh probabilities since palaeobotanical proofs are not available. However, Heim quotes me as having said in 1936 that "there is only one line of descent of Agaricales from Gastromycetes." Nowhere and never have I said or thought this; what I have said in the paper quoted reads (in English translation): "The Agaricales are not polyphyletically derived in the sense that some descend from the Gastromycetes and some from the Aphyllophorales; they are well separated from the latter and represent the hemiangiocarpous, pseudoangiocarpous and gymnocarpous, epigeous, non-xerophytic continuations of phylogenetic lines starting from various lines of Gastromycetes which have reached a certain level of structural organization. . . ." This was said after it was explained that the sentence just quoted would be the conclusion most adapted to our present knowledge, if I should succeed in rejecting all the so-called links supposedly existing between Agaricales and other groups of Basidiomycetes (these links were enumerated on the previous page). Since I did not say what I am supposed to have said, Heim's sentence, "The hypothesis of our friend R. Singer seems to be refuted by these new arguments also," can only apply to what I have not said rather than to what I have stated. I wonder if it was not difficulties in translation which led to this misunderstanding.

The new arguments which allegedly refute my hypothesis are mostly those indicated in an earlier paper by Kühner who certainly did not go so far as to state that they refute my ideas but merely indicated them as being in favor of Fayod's opinions on phylogeny rather than of mine. However, these data are of questionable value when applied in favor of a certain phylogenetical theory. It is, of course, true that the species with clamp connections are in principle more ancient than those without clamps in the same line of evolution. Since Heim believes that I derive the agarics monophyletically, he may also believe that the fact that there are many clamped species without veils and *vice versa* is in contradiction

with this statement. The same is true—if indeed the binucleate spores are a more recent development in the phylogeny of the Agaricales than the uninucleate ones—in regard to the argument that *Amanita* has binucleate spores while *Clitocybe* has uninucleate ones. Let us, however, not forget that *Catathelasma*, with a strongly developed double veil, has uninucleate spores and clamp connections! Gymnocarpous development is also not necessarily an argument against derivation from Gastromycetes. It is theoretically quite possible that a gastroid form like *Torrendia* with simple-walled hyaline spores, has acquired gymnocarpous development and various other "recent" characteristics before reaching the stage where the decisive transformation took place, *i.e.* the transformation into an organism we might call an agaric. Heim's own argument concerning the carotene crystals discovered by him in phalloids is correct insofar as it restates the generally high organization of the phalloids among the basidiomycetes. This statement is at present accepted by many authors, and probably rightly so, but has nothing whatever to do with refuting the derivation of agarics from gastromycetes. The branching off of the agarics and boletes must have taken place at various levels and along different lines—all completely unrelated to anything referring to the Phallales.

As for the positive value of the veils in competition with naked species in nature, I have still found no shadow of a proof. Heim says it is explained in his paper on *Termitomyces* (**1941**) which, as it is an excellent paper with numerous valuable new facts and considerable thoroughness, has been studied by me thoroughly and repeatedly, but without furnishing any data supporting the ancient theory on the usefulness and protective function of the veil counterbalancing the obviously negative effect it must have on spore dissemination in species depending for spore dispersal on air currents and wind.

Since there is, up to this day, no forceful argument against derivation of the Agaricales from various Gastromycetes, we may now examine the possibility of a derivation of the Cortinarii from *Thaxterogaster*. The only members of the Cortinariaceae without clamp connections are two species of one section of *Galerina* which,

in comparison with *Cortinarius*, is evidently a more recent genus, more highly organized and more specialized (cheilocystidia or cystidia occurring in all species, more special adaptations to various epigeous habitats than in *Cortinarius*). Both *Thaxterogaster* species have clamp connections. I have not studied the spores cytologically but since those of the Cortinariaceae are binucleate, they could hardly be anything but either of the same type, or uninucleate. Since the veils are not essentially more strongly developed in *Thaxterogaster* than in *Cortinarius*, only more persistent, the formation of veils does not enter this discussion. So much for the arguments used by Kühner. Another argument that might perhaps play a rôle in the discussion of *Montagnea* and *Galeropsis* in their relation to *Coprinus* and *Cyttarophyllum*—the fact that the gastroid forms are xerophytic, which is usually a more recent adaptive condition—cannot enter the discussion about *Thaxterogaster* since the latter is a typical fleshy-putrescent hygrophilous species of the forest humus. In favor of a theory considering *Thaxterogaster* as the form from which the agarics originated is the tendency of the Cortinarii in Tierra del Fuego to form aborted hymenophores, a fact that cannot by any stretch of the imagination be considered as a progressive, "useful" development but must be explained as an atavistic tendency brought about by conditions favoring local detrimental mutations. The small number of species of *Thaxterogaster*, a genus with either one small area, or with isolated small areas, as compared with the large genus *Cortinarius* with continuous distribution in all continents, with small units often difficult to separate taxonomically, makes it extremely difficult to assume a direction of evolutive tendencies starting from *Cortinarius* and leading to *Thaxterogaster*. The similarity of *Thaxterogaster* in its mature form with *Cortinarius* in its immature form would also tend to corroborate the opposite view rather than the classical view, defended by Fayod.

If we keep in mind that the structure of the spores seems, as a general rule, to remain virtually the same during evolutionary processes leading from the gastroid to the agaricoid form (or *vice versa*), one should not be surprised to see the same rule corroborated in the case of *Thaxterogaster*. There is no tendency towards a germ-pore development, or towards complex walls, or

towards binucleate spores during the process of transformation from the gastroid to the agaricoid form (or *vice versa*), but this tendency may have played a certain rôle at a much earlier stage of phylogenetic development. Locquin showed that the germination of agaric spores does not as a rule take place at the point of the germ pore; hence the presence of the germ pore in thick-walled spores may be more of a genotypic character without much biological importance at the agaric level. As for the agarics with thin-walled spores of a simple structure and predominantly one nucleus at the time of discharge, we must most probably look for an ancestor quite different from those giving origin to the Bolbitiaceae, Coprinaceae and Cortinariaceae, indeed a species with the same—possibly lower—organization of spore structure. However this may be, the spore structure of *Thaxterogaster* does not seem to give any indication as to the possible direction of evolution unless one considers the large spores of *Thaxterogaster* as another argument against their comparatively recent origin since the similar Cortinarii, especially those with gastroid hymenophore-aberrations in Tierra del Fuego, have, on an average, much smaller spores, and large spores should, on principle, and within the same group, be regarded as an indication of ancient forms, relict forms, or generally forms from which small-spored forms have taken their origin.

SUMMARY

1. A new genus and two new species of this new genus are reported from Tierra del Fuego. This genus is here called *Thaxterogaster*, as Thaxter was the first to collect a representative of this interesting genus in the western hemisphere (Punta Arenas, Chile).

2. *Thaxterogaster* is a genus of the Secotiaceae Corda, a true Gastromycete.

3. *Thaxterogaster* has close affinity with the Cortinariaceae, especially the genus *Cortinarius* Fr.

4. There are some data which tend to favor the assumption that *Cortinarius* is derived from *Thaxterogaster* rather than *vice versa*. These data are corroborated by certain observations at the type locality.

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SOME NEW SPECIES OF DISCOMYCETES FROM MOUNT SHASTA

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Among the numerous fungi collected on Mount Shasta, California, during recent years by Wm. Bridge Cooke, various specimens of Discomycetes have been referred to the writer for examination. Several of these do not appear to agree with described species and are therefore named here as new. Type specimens are deposited in the Mycological Collections of the Bureau of Plant Industry, Soils and Agricultural Engineering, and duplicates are in Dr. Cooke's herbarium at Pullman, Washington.

1. **Dasyscypha aplopappi** sp. n.

Apothecia sessilia, depresso-globosa, ore parvo rotundo et margine inflexo, carnosa, usque 1 mm. in diam. et altitudine, dense brunneo-tomentoso, hymenio flavido-griseo; asci subsessiles, teretes, 80–95 × 10–11 μ, 4–8-spori, ad apices rotundati, subapplanati incrassatulique; paraphyses eramosae, tenues, hyalinae, ascos superantes, ad apices subacutae, 3–4 μ; ascosporae ellipsoideae, hyalines, unicellulares, 1–2-seriatae, 14–17(–22) × 4–5 μ; pili flavo-brunnei, septati, verrucosi, 150–200 × 3.5–4 μ.

Apothecia superficial, sessile, single, depressed-globose, fleshy, up to 1 mm. in diameter and height, densely brown tomentose, remaining unexpanded, with small round opening and inrolled or folded margin, Prout's brown [1] to mummy brown, hymenium drab gray; asci subsessile, terete, 80–95 × 10–11 μ, usually 8-, occasionally 4-spored, rounded at the apex and the wall slightly flattened and thickened; paraphyses unbranched, septate, hyaline, slender, flexuous, narrowed toward the subacute tips, longer than the asci, 3–4 μ thick at the broadest point; excipular hairs 150–200 × 3–4 μ, septate, yellow-brown, verrucose, even or very slightly swollen at the hyaline tips; ascospores ellipsoid, hyaline, one-celled, uni- or biseriate, 14–17(–22) × 4–5 μ.

On decorticated twigs of *Aplopappus bloomeri* var. *angustatus*, Horse Camp, Mt. Shasta, Calif., 8000 ft., June 27 and July 7 and

[1] Color readings made from Ridgway, R., Color standards and color nomenclature, Washington, 1912.

8, 1946, W. B. Cooke *18082, 18175* and *18180,* **type**; between Panther Creek and Wagon Camp, 7000 ft., Aug. 15, 1947 *20437.*

The paraphyses of this fungus are more acute than those in *Dasyscypha flammea* (Alb. & Schw. ex Fr.) Dennis, which it somewhat resembles. It differs from that species also in the pallid hymenium and brown hairs and from *Lachnum fusco-floccosum* Rehm and *Dasyscypha calyculiforme* (Schum. ex Fr.) Rehm in larger spores.

2. Dasyscypha phlogis sp. n.

Apothecia dispersa, brevi-stipitata, subglobosa vel turbinata, ore minuto et margine fimbriato inflexo, dense brunneo-tomentosa, 0.5 mm. diam. et altitudine; stipite 100–150 μ longo et crasso; asci cylindrici, ad apices et pedicellos breve attenuati, 55–60 × 6–7 μ; ascosporae fusoideo-ellipsoideae, hyalinae, unicellulares, 10–12 × 1.5–2.5 μ; paraphyses eramosae, subacutae, angustae, 60–75 × 2–3.5 μ; pili flavo-brunnei, echinulati, septati, 100–125 × 4–5 μ, ad apices pallidiores subincrassati, crystalligeri.

Apothecia scattered, short-stipitate, fleshy, cinnamon brown to Prout's brown, subglobose to turbinate, opening small, margin fimbriate, remaining tightly inrolled and concealing the hymenium, densely brown-hirsute, 0.5 mm. in diameter and height, stipe 100–150 μ long and thick; asci cylindrical, narrowed at the apex and to a short pedicel, apical pore blue with iodine; ascospores fusoid-ellipsoid, hyaline, unicellular, obliquely uniseriate below to irregularly biseriate above, 10–12 × 1.5–2.5 μ; paraphyses unbranched, narrow, subacute, 60–75 × 2–3.5 μ; exciple hyaline, prosenchymatous, excipular hairs yellow-brown, echinulate, 100–125 × 4–5 μ, septate, paler and slightly swollen at the tips, often bearing hyaline crystals.

On stems of *Phlox douglasii* (living plants), south of Horse Camp, 7750 ft., Aug. 27, 1947, W. B. Cooke *20484,* **type.**

No species of *Dasyscypha* has been found reported on this host. *D. phlogis* differs from most other caulicolous species of the genus in its minute size.

3. Dasyscypha salmonea sp. n.

Apothecia carnosa, sessilia, subglobosa dein cupulata margine crenata, sicca inflexa plicataque, 0.5–1.2 mm. in diam., pallide incarnata, hymenio flavidulo, sicco salmoneo; asci cylindrici usque longe cylindrico-clavati, longe pedi-

cellati, apices rotundatos versus angustati, 75–78 × 6–7.5 μ; ascosporae unicellulares, anguste ellipsoideae, hyalinae, 1–2-seriatae, 11–15 × 3 μ; paraphyses numerosae, filiformes, eramosae, dense granulosae, 1 μ crassae; excipulum prosenchymaticum, subhyalinum, pilis hyalinis, granulosis, septatis, usque 3.5 μ in diam. ad marginem fasciculatis vestitum.

Apothecia fleshy, sessile, subglobose then cupuliform, margin crenate, inrolled and plicate when dry, 0.5–1.2 mm. in diameter, pale flesh color to seashell pink, hymenium light orange yellow, drying flesh color to salmon buff; asci cylindrical or long cylindrical-clavate, long-pedicellate, slightly narrowed toward the rounded tips, wall thickened at the apex; 75–78 × 6–7.5 μ; ascospores unicellular, narrow-ellipsoid, hyaline, 1–2-seriate; paraphyses numerous, filiform, unbranched, filled with dense orange granules, 1 μ thick; exciple prosenchymatous, subhyaline; hairs hyaline, granulose, septate, up to 3.5 μ in diameter thick, fasciculate at the margin.

On dead decorticated branches of low shrubs of *Aplopappus bloomeri* var. *angustatus*, Horse Camp, 8000 ft., Mt. Shasta, Calif., June 27 and July 7, 1946, W. B. and V. G. Cooke, no. *18081* and *18174*, Horse Camp, July 8, 1946, *18179*, **type**; above Horse Camp, 8250 ft., Sept. 28, 1950, W. B. and V. G. Cooke *27015*.

The salmon pink apothecia distinguish the fungus from the species of the genus occurring on stems and branches. Among specimens approaching it in color, *D. fairmani* Rehm has smooth brown excipular hairs; the spores on *Lachnella campanula* Ell. are apiculate and *D. incarnata* Clements is long-stipitate.

4. Laetinaevia veratri sp. n.

Apothecia sessilia, e fibris hospitis partim emergentia, dense aggregata, carnosa, pallide carneo-ochracea, sicca rubra, cupulata vel patellata, 0.2–1.5 mm. in diam., in ambitu circularia vel elliptica, margine albo-fimbriato; asci clavati, ad bases attenuati, pariete ad apices incrassata, 75–80 × 12–13 μ; ascosporae cylindricae, obtusae, 1–2-cellulatae, hyalinae vel subhyalinae, multiguttulatae, 13–15 × 2 μ; paraphyses numerosae, filiformes, hyalinae, eramosae, ad apices undulatas 2 μ in diam.; excipulum subhyalinum, ad basim pseudoparenchymaticum, marginem versus prosenchymaticum.

Apothecia sessile, only partially emerging from between fibers of host, densely gregarious, fleshy, pale flesh-color or pale ochraceous, drying carrot red to carnelian red, cupulate to patellate, 0.2–1.5 mm., round to elliptical in outline, margin whitish-fimbriate; asci clavate, attenuated toward the base, slightly narrowed and wall

thickened at the apex, 75–80 × 12–13 μ; ascospores cylindrical, straight or slightly curved, ends obtuse, 1–2-celled, hyaline or subhyaline, multiguttulate, 13–15 × 2 μ; paraphyses numerous, filiform, hyaline, unbranched, the tips undulate, and slightly thickened to 2 μ; exciple subhyaline, pseudoparenchymatic at base, composed of thin-walled hexagonal cells 6–9 μ in diam., becoming prosenchymatic at margin.

On *Veratrum californicum*, Wagon Camp, Mt. Shasta, Calif., 5700 ft., June 21, 1946, W. B. Cooke *18009*, **type**; Crater Lake Nat. Pk., Oregon, July 5, 1948, 6250 ft., W. B. and V. G. Cooke *24095*.

This fungus appears to have the essential characters of *Laetinaevia*, a genus which includes several species originally referred to *Orbilia* or *Calloria*. Several species of *Calloria* described on herbaceous stems are somewhat similar, but none appears to agree completely. The spores are smaller in *C. caulophylli* (Ell. & Ev.) Rehm and *C. solidaginis* Kanouse, while in *C. oleosa* (Ell.) Sacc. and *C. subalpina* Rehm they are fusoid. Examination of type material of *C. subalpina* (Krieger F. Saxonici no. *2164*) furthermore shows branched paraphyses, swollen abruptly at the apices and agglutinated into a mazaedium.

The specimen from Crater Lake National Park, Oregon, is referred to this species with some doubt. The apothecia in the Oregon material are similar in appearance and color, although smaller and more scattered. Free spores resembling those of the type were present, although the few spores found in asci were slightly broader than those in the Mt. Shasta specimen.

5. Mollisia shastensis sp. n.

Apothecia sessilia, molle, 0.5–0.8 mm. diam., carnosa, patellata, rubro-brunnea, sicca nigro-brunnea, hymenio griseo-avellaneo, margine involuto et fimbriato; asci cylindrici, gradatim apicem basimque versus attenuati, 50.6–63.8 × 5–7 μ; ascosporae hyalinae, ellipsoideo-clavatae, unicellulares, 1–2-seriatae, saepe 2-guttulatae, 6–8 × 1.5–2 μ; paraphyses filiformes, hyalinae, rare base ramosae, ad apices vix usque 1.5 μ inflatae; excipulum pseudoparenchymaticum, cellulis brunneis hexagonis compositum, marginem fimbriatum versus in prosenchymatem exeunte; hyphae marginales subhyalinae, asperatulae, in caespitulis intertextae, hyphae basilares paucae, pallide brunneae, 2–3 μ crassae.

Apothecia sessile, 0.5–0.8 mm. in diameter, soft-fleshy, patellate, natal brown to bone brown, drying black, hymenium light drab to drab, margin involute, fimbriate; asci cylindrical, gradually attenuated toward the apex and base, 8-spored, 50.6–63.8 × 5–7 μ; ascospores ellipsoid-clavate, often 2-guttulate, hyaline, 6–8 × 1.5–2 μ; paraphyses filiform, hyaline, unbranched or branched near the base, scarcely inflated at the apex to 1.5 μ; exciple pseudoparenchymatic, made up of brown hexagonal cells, becoming prosenchymatic toward the margin, with clumps of tangled, subhyaline to pale brown, slightly roughened hyphae; basal hyphae scanty, pale brown, 2–3 μ thick.

On dead roots of *Eriogonum pyrolaefolium*, above Horse Camp, 8500 ft., Mt. Shasta, Calif., Sept. 4, 1947, W. B. Cooke *20605*, **type**; on stems of *Arctostaphylos nevadensis*, between Horse Camp and Panther Creek Meadows, 7500 ft., Aug. 15, 1947, W. B. Cooke *20456*.

The spores of *M. shastensis* are shorter and the apothecia darker than those of *M. melaleuca* (Fr.) Sacc.

6. **Mollisia hysteroides** sp. n.

Apothecia sessilia, superficialia, carnosa, singula, patellata, 0.7–1.2 mm. diam., sicca hysteroidea plicata margine stricte involuto, fusco-nigra, hymenio pallide griseo usque ardesiaco; asci teretes, gradatim pedicellum brevem versus attenuati, ad apices late rotundati, octospori, 65–75 × 7–8 μ; ascospores hyalinae, unicellulares, longe ellipsoideae, rectae vel suballantoideae, 12–14 × 3–3.5 μ; paraphyses filiformes, eramosae, ad apices subglobosas vel obpyriformes, flavo-brunneas, usque 3–3.5 μ diam. abrupte inflatae; excipulum pseudoparenchymaticum, e cellulis dense compactis crasse-tunicatis hexagonis 7–10 μ diam. compositis, ad marginem in hyphas pallidiores flavo-brunneas exeunte.

Apothecia sessile, superficial, fleshy, single, patellate, 0.7–1.2 mm. in diameter, hysteroid and nearly black when dry, with margin tightly inrolled, fuscous black, hymenium pale neutral gray to slate color or blackish slate; asci terete, gradually narrowed to a short pedicel and broadly rounded at the apex, 8-spored, 65–75 × 7–8 μ; ascospores hyaline, unicellular, long-ellipsoid, straight or suballantoid, 12–14 × 3–3.5 μ; paraphyses filiform, unbranched, abruptly swollen to subglobose or obpyriform, apices golden brown, 3–3.5 μ in diameter; exciple of densely compact thick-walled hexagonal cells 7–10 μ in diameter, changing to paler yellowish-brown hyphae toward the margin.

On stems of *Aplopappus bloomeri* var. *angustatus*, Horse Camp, Mt. Shasta, Calif., July 7, 1946, Wm. B. Cooke *18176* and July 8, 1946, *18178*, **type**; on *Phlox douglasii*, Horse Camp, July 7, 1946, W. B. Cooke *18173*.

In the longitudinal folding and clavate paraphyses, this species resembles *M. tyrolensis* Sacc.; the latter, however, has smaller apothecia, asci and spores, and differs also in its reddish brown color.

U. S. PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND

NEW AGARICS FROM FLORIDA

W. A. Murrill

Clitocybe subfellea sp. nov.

Pileo convexo-expanso, umbonato, 5 cm. lato, glabro, pallido, felleo; lamellis decurrentibus, confertis, albis; sporis ovoideis, levibus, 5–6 × 2.5–3 μ; stipite solido, glabro, albo, 5 × 0.4–0.5 cm.

Pileus convex to expanded with broad umbo, scattered, about 5 cm. broad; surface smooth, glabrous, white or slightly yellow, margin at first incurved, undulate or somewhat lobed, even, concolorous; context to 4 mm. thick, white, unchanging, bitter, with anise odor; lamellae arched, 4 mm. broad, crowded, inserted, decurrent, entire, thin, white, unchanging, not fimbriate; spores ovoid, smooth, hyaline, obliquely apiculate, uniguttulate, about 5–6 × 2.5–3 μ; stipe solid, firm, slightly enlarged above and below, smooth, white, glabrous, about 5 × 0.4–0.5 cm.

Type collected by G. F. Weber in mixed woods on the shore of Lake Rosa, Putnam Co., Fla., Oct. 13, 1947 (*F 19337*). Locally plentiful then but not found elsewhere.

Collybia umbrinescens sp. nov.

Pileo umbilicato, gregario, 4.5 cm. lato, glabro, subisabellino, subfarinaceo; lamellis sinuatis, 8 mm. latis, albis; sporis oblongo-ellipsoideis, levibus, 6 × 3 μ; stipite glabro, pallido, 6 × 0.4–0.7 cm.

Pileus umbilicate, gregarious, about 4.5 cm. broad; surface smooth, glabrous, uniformly pale-isabelline, margin even, entire, deflexed; context thin, white, unchanging, odorless, taste slightly farinaceous; lamellae plane, sinuate, medium close, broad behind, reaching 8 mm., edges white, uneven; spores oblong-ellipsoid, inequilateral, hyaline, smooth, about 6 × 3 μ; stipe tapering downward, smooth, glabrous, pallid with a faint rosy-isabelline tint, 6 × 0.4–0.7 cm.

Type collected by G. F. Weber about a cypress pond in Cary Forest, east of Gainesville, Fla., Dec. 30, 1945 (*F 19358*). Found but once. After drying the entire hymenophore becomes um-

brinous. In my nomenclature this would be **Gymnopus umbrinescens.** It is an anomalous species and has no near relatives.

Coprinus Westii sp. nov.

Pileo cylindrico-campanulato, gregario, 10 × 13 cm., albo, imbricato-squamoso; lamellis confertis, angustatis, albis; sporis ellipsoideis, levibus, 20–25 × 12–14 μ; stipite aequali, radicato, glabro, albo, 20 × 1.5–2 cm.; annulo nullo.

Pileus cylindric to campanulate, gregarious, 10 cm. high and 13 cm. broad; surface white, densely imbricate-scaly, margin entire to ragged, concolorous; context thin, white, odorless; lamellae narrow, crowded, white, at length black and melting; spores ellipsoid, black, smooth, 20–25 × 12–14 μ; stipe equal, radicate, smooth, glabrous, white, unchanging, about 20 × 1.5–2 cm.; annulus none.

Type collected by Erdman West on the ground in mixed woods at Warren's Cave, northwest of Gainesville, Fla., Oct. 27, 1946 (*F 25778*). Found but once. Suggesting *C. praemagnus* Murr. but differing in several important characters.

Entoloma Weberi sp. nov.

Pileo convexo-plano, umbonato, 7 cm. lato, glabro, avellaneo-umbrino, farinaceo, demum felleo; lamellis subconfertis, 8 mm. latis; sporis angulatis, 10 × 6 μ; stipite subaequali, glabro, subavellaneo, 9 × 0.7–0.9 cm.

Pileus convex to plane with conic umbo, gregarious, about 7 cm. broad; surface faintly striate and checked, glabrous, avellaneous-umbrinous, margin thin, straight, becoming somewhat lobed or split, concolorous; context thin, white, unchanging, odor farinaceous, taste farinaceous to bitter; lamellae sinuate, rather crowded, plane when young, becoming 8 mm. broad behind with age, edges uneven; spores distinctly angular, elliptical in outline, about 10 × 6 μ; stipe subequal, twisted, smooth, glabrous, pale-avellaneous, the base white, about 9 × 0.7–0.9 cm.

Type collected by G. F. Weber about a cypress pond in Cary Forest, east of Gainesville, Fla., Dec. 30, 1945 (*F 19370*). Locally abundant at the time but not found since. Its nearest relative is probably *E. Cokeri* Murr., described from Chapel Hill, N. C.

Lepiota aspericeps sp. nov.

Pileo convexo-plano, umbonato, 1.5 cm. lato, squamis hirtis obsito, roseo-isabellino, umbone badio; lamellis latis, confertis, fimbriatis; sporis ovato-

oblongis, levibus, 6–7 × 3 μ; stipite aequali, glabro, concolori, 4 × 0.1 cm.; annulo supero, albo, margine brunneo.

Pileus convex to plane with a tiny conic umbo, gregarious, 1.5 cm. broad; surface dry, rosy-isabelline, beset with sharp, rigid spines, which are more prominent on the bay umbo, margin thin, even, concolorous, somewhat ragged; context very thin, white, odorless; lamellae free, broad, ventricose, inserted, crowded, white, unchanging, fimbriate; spores pip-shaped, smooth, uniguttulate, about 6–7 × 3 μ; stipe equal, smooth, glabrous, rosy-isabelline, about 4 × 0.1 cm.; annulus superior, fixed, persistent, white, brown on the edge.

Type collected by W. A. Murrill on the ground in a high hammock at Gainesville, Fla., Sep. 25, 1944 (*F 10447*). Found but once.

Lepiota murinidisca sp. nov.

Pileo convexo-expanso, submammillato, 2 cm. lato, squamuloso, atrogriseo; lamellis latis, ventricosis, fimbriatis, albis; sporis ovato-oblongis, levibus, 5–7 × 3–4 μ; stipite aequali, glabro, albo, 2.5 × 0.15 cm.; annulo supero, albo.

Pileus convex to expanded, slightly mammillate, gregarious, 2 cm. broad; surface dry, faintly striate, minutely scaly, dark-griseous with murinous disk, margin entire, concolorous; context thin, white, odorless; lamellae broad, ventricose, medium close, white, unchanging, the edges fimbriate; spores oblong-ovoid, smooth, about 5–7 × 3–4 μ; stipe equal, smooth, glabrous, white, 2.5 × 0.15 cm.; annulus superior, fixed, persistent, white with dark border.

Type collected by W. A. Murrill on a rich bank shaded by a variety of trees in Gainesville, Fla., July 31, 1948 (*F 21111*). Not found elsewhere.

Lepiota neglecta sp. nov.

Pileo conico-expanso, 1–5 cm. lato, striato, albo, squamuloso, centro fusco, grato; lamellis latis, confertis, albis; sporis ellipsoideis, levibus, 5 × 3 μ; stipite glabro, albo, demum roseo-isabellino, 3 × 0.1 cm.; annulo amplo, albo.

Pileus subconic to subexpanded, scattered, about 1.5 cm. broad; surface dry, striate, excoriate, white, with blackish to fuliginous disk and scales, margin entire, at length somewhat ragged; context membranous, white, unchanging, with pleasant taste, odor not characteristic; lamellae broad, crowded, white, unchanging, free, minutely fringed on the edges; spores ellipsoid, smooth, hyaline, about

5 × 3 μ; stipe stuffed, tapering upward, smooth, glabrous, white to rosy-isabelline, about 3 × 0.1 cm.; annulus median, ample, skirt-like, usually persistent, white.

Type collected by W. A. Murrill under a live-oak, scattered in short grass, at Gainesville, Fla., Aug. 8, 1944 (*F 15780*). Rare in the vicinity. In herbarium specimens, the umbo at length becomes dark-bay.

Leptonia domestica sp. nov.

Pileo convexo-plano, 2–4 cm. lato, striato, avellaneo, grato; lamellis adnexis, latis, distantibus, albis; sporis praeangulatis, 9 × 7 μ; cystidiis nullis; stipite aequali, glabro, subfumoso, 3–4 × 0.2–0.4 cm.

Pileus convex to plane, gregarious, 2–4 cm. broad; surface striate, fibrillose, avellaneous with darker center, margin at first incurved, entire to ragged; context thin, white, odorless, mild; lamellae adnexed, inserted, broad, distant, white, entire; spores decidedly angular, uniguttulate, about 9 × 7 μ; cystidia none; stipe equal, flat, hollow, smooth, glabrous, pale-fumose, 3–4 × 0.2–0.4 cm.

Type collected by W. A. Murrill on an open grassy lawn in Gainesville, Fla., Aug. 9, 1944 (*F 32681*). Found but once. In my nomenclature this would be **Leptoniella domestica**.

Leptonia subfloridana sp. nov.

Pileo convexo-subdepresso, 2–2.5 cm. lato, glabro, sublucido, fumoso-avellaneo, praefarinaceo; lamellis sinuatis, subdistantibus, pallidis, demum subisabellinis; sporis angulatis, 9 × 7 μ; stipite glabro, avellaneo, 4 × 0.3 cm.

Pileus convex to slightly depressed, 2–2.5 cm. broad; surface smooth, glabrous, shining, fumose-avellaneous, margin inflexed, entire, even, concolorous; context thin, white, with strong farinaceous taste and odor; lamellae sinuate, inserted, rather distant, uneven, pallid to pale-isabelline; spores decidedly angular, ellipsoid in outline, uniguttulate, about 9 × 7 μ; cystidia none; stipe smooth, glabrous, avellaneous, whitish at apex and base, about 4 × 0.3 cm.

Type collected by W. A. Murrill on the ground in Kelly's Hammock, northwest of Gainesville, Fla., July 19, 1938 (*F 19382*). Found but once. Suggesting *L. floridana* Murr. but with farinaceous odor and taste. In my nomenclature this would be **Leptoniella subfloridana**.

Nolanea mammillata sp. nov.

Pileo conico-campanulato, umbonato, 3 cm. lato, striato, subavellaneo, praefelleo; lamellis adnexis, confertis, 6 mm. latis, albis; sporis angulatis, 10–12 × 6–8 μ; stipite aequali, glabro, pallido, 5 × 0.3–0.4 cm.

Pileus conic to campanulate with prominent mammillate umbo, 3 cm. broad; surface satiny, striate, pale-avellaneous, the umbo smooth and pallid, margin undulate, split with age, brownish; context very thin, soft, odorless, very bitter at once; lamellae adnexed, inserted, broad and rounded behind, close, the short ones narrower, undulate to slightly eroded, white to old-rose; spores decidedly angular, 10–12 × 6–8 μ; cystidia none; stipe equal, twisted, glabrous, longitudinally striate, dull, pallid, whitish-mycelioid at base, about 5 × 0.3–0.4 cm.

Type collected by W. A. Murrill by a pond in low mixed woods in Sugarfoot Hammock, near Gainesville, Fla., Sep. 17, 1944 (*F 18060*). Found but once. Near *N. pallidiceps* but very bitter.

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REVIEWS

THE STIPITATE HYDNUMS OF THE EASTERN UNITED STATES, by William Chambers Coker and Alma Holland Beers. i–viii + 86 pp., 60 plates. The University of North Carolina Press, Chapel Hill, North Carolina. 1951. Price $5.00.

This volume is another in an impressive series of books by Coker and his associates. The standard set in *The Boletaceae of North Carolina,* and other books and papers, has been maintained here.

Over a period of more than thirty years, the senior author has been studying and publishing on the Hydnaceae. As the authors state in the Preface, this book "represents a reworking of all this material, together with many revisions and additions, incorporating our notes accumulated during and since that period." In further preparation for this publication, Dr. Coker has visited important herbaria in this country and in Europe, and thus has been privileged to compare a large number of collections in the group treated.

The authors begin their book with a key to the genera treated. This key is then supplemented by a composite key to the fleshy, stipitate Hydnums of the eastern United States. Following generic characterization, keys to the species of each genus are appropriately presented. The use of these keys by other mycologists working with fresh material will test their adequacy more completely.

In the area covered (Eastern United States and, to some extent, Eastern Canada), ten genera are recognized, one of them new: *Sistotrema, Steccherinum* (including both stipitate and sessile species), *Auriscalpium, Hydnum, Phellodon, Bankera, Sarcodon, Hydnellum, Pseudohydnum,* and *Hericium.* The generic concepts adhered to are generally orthodox, and include texture of the sporophore, spore color and surface markings, and, to a less extent, habitat. Primary separation, in the key to genera, is based on the presence or absence of a distinct pileus and stipe.

The new genus recognized is designated as **Bankera.** It is based on *Hydnum fuligineo-album* Schmidt (*Sarcodon reticulatus*

Banker), and but one species (*Bankera fuligineo-alba*) is reported. The new genus is characterized chiefly by white, tuberculate spores, and fleshy, homogeneous context throughout.

For each of the sixty species treated, the authors present rather full description, followed by discussion which is usually brief and is designed to supplement the description. Illustrations of other workers are cited, and distribution notes are recorded for the United States, Canada, and Europe.

The authors have elected to conform to the American Code of nomenclature. Their manual is then made the more usable by a listing of common synonyms together with customary citations.

In addition to the proposed new genus, *Bankera*, two new species are described, *Phellodon* **brunneo-olivaceus** and *Sarcodon* **excentricus**; and one new form, *Hydnellum diabolus* forma **reticulatum**. Descriptions of these new plants are in English. Some of us would have been mildly surprised if the authors had presented their diagnoses in Latin.

The composition of the book is excellent, the photographs well selected, and the line drawings expertly done. An index and an adequate bibliography of some fifty references complete this welcome volume.—L. R. HESLER.

THE ACTINOMYCETES, THEIR NATURE, OCCURRENCE, ACTIVITIES AND IMPORTANCE, by Selman A. Waksman. pp. i–xviii, 1–230. 38 ill. Annales Cryptogamici et Phytopathologici, Vol. 9. Waltham, Mass. The Chronica Botanica Co. 1950. Price $5.00.

In this book Dr. Waksman has brought together and combined with his own experience information from some five hundred publications relating in diverse ways to the actinomycetes. This material is organized into twelve chapters covering a range of interest from taxonomy and morphology, through physiology and antibiotic production, to the activities of the actinomycetes in the soil and as agents of plant and animal disease. Although most of the subject matter is available in the literature, inclusive of the author's own articles and books, some of it is not readily accessible and Dr. Waksman has performed a service in providing a convenient source of information on the actinomycetes.

It is disappointing to those who prior to publication of The Actinomycetes had hoped the book would include a more adequate and detailed taxonomic treatment of the group than Bergey's Manual can offer, to find that the taxonomic treatment is limited to the characterization of the order, its two families and four genera, and descriptions of only seven species. It is apparent, though, that a monographic treatment of the actinomycetes, however badly needed, was not the purpose of this book and, indeed, to hope for such at this time may be premature. The author ably describes in his chapter on taxonomy the confusion existing in the classification of the actinomycetes prior to the introduction of the classification of Waksman and Henrici in 1943. The fact that Waksman can list twenty-three different names as the more common synonyms of the four genera of the Actinomycetales presently recognized, is evidence of the extremely controversial and complex state of actinomycete taxonomy from 1875 to the present time.

The book is well illustrated with photographs, of which those by Littman of the Armed Forces Institute of Pathology should be given special notice for their excellence. The microscopic structures of the actinomycetes are notoriously difficult to photograph and therefore, the author should be commended for the photographs comprising in particular figures 5 and 12.

Because recent interest in the antibiotic-producing abilities of the actinomycetes has stimulated work on the morphology and physiology as well as on the antagonistic properties of these organisms, Waksman draws heavily from the antibiotic literature in his chapters on morphology, variation and mutation, metabolism, and antibiotics of the actinomycetes. The chapter on antibiotics includes a useful key to the antiobiotics produced by the actinomycetes.

The appendix contains a list of thirty-one culture media for the study of actinomycetes and a bibliography of 522 references. Both a subject index and an index to organisms are provided.—ALMA J. WHIFFEN.

THE BRITISH SMUT FUNGI, by G. C. Ainsworth and Kathleen Sampson. 137 pp., 2 pls., 21 figs. Commonwealth Mycological Institute, Kew, Surrey. 1950. Price $3.00.

Those of us who knew that Dr. Ainsworth and Miss Sampson had been preparing an account of British smut fungi have been waiting impatiently for its appearance. When *The British Smut Fungi* came off the press recently we were not disappointed.

The British Smut Fungi is well organized to include sections on Biology, Cytology, Genetics, and Technique, in that order, followed by a systematic treatment of the smut fungi of the British Isles.

Following a brief Introduction, which covers the general characteristics of smut fungi, their economic importance, and occurrence in Britain, the authors devote 16 pages to "Biology." They describe in rather condensed form the phenomena of penetration, invasion, and parasitism in general. These sub-topics are followed by a short account of chlamydospore formation and of the various types of chlamydospore germination and the factors influencing this process. The development of sporidia on the host is discussed. The section on biology is concluded with two and one-half pages devoted to "Growth in Culture." The characters of monosporidial cultures, monospore cultures, "monocaryotic" and "dicaryophytic" mycelia, and the development of sporidia and chlamydospores in culture are summarized.

The section on "Cytology" contains a good, brief digest of the contributions in the major papers on cytology of the smut fungi.

The section on "Genetics" is more extensively developed. Sex or compatibility groups are discussed first, under the heading "Incompatibility." This is followed by a short account of "The Gametophyte in Culture" and others on "Spore and Soral Characters," "Germination of Hybrid Spores" and "Pathogenicity." The section is concluded with a more comprehensive treatment of "Mutation" in cultural characters of smut fungi.

One of the most useful of the sections preceding the systematic treatment is that on "Technique." In this section is given first of all brief directions for "Collection and Examination of Herbarium Material," followed by techniques for "Harvesting, Storage, and Germination of Chlamydospores." In the very short account of "Media for Growth in Culture," some of the more common agar media are given. The techniques for obtaining monosporidial cultures are presented, followed by "Tests for the Compatibility of

Monosporidial Lines." Three and one-half pages are then given to the several inoculation methods under the heading "Infection of the Host." Control techniques are next very briefly discussed, followed by "Fixatives." The section is concluded with an account of staining techniques.

In the next sixty pages there is presented a systematic account of the British smut fungi. Three families are recognized: the usually included Ustilaginaceae and Tilletiaceae, and, in addition, the sometimes excluded Graphiolaceae. The Ustilaginaceae is represented by the genera *Ustilago, Farysia, Sphacelotheca, Cintractia,* and *Thecaphora,* of which the largest number of species (23) are in *Ustilago.* The species of this genus are "grouped according to whether the spores are smooth or granular, verrucose or echinulate, or reticulate, and they are arranged in increasing spore size within each group." The Tilletiaceae is represented by species of *Tilletia, Entorrhiza, Schroeteria, Tuburcinia, Urocystis, Melanotaenium, Entyloma,* and *Doassansia.* The greater numbers of British species are in *Urocystis* and *Entyloma* with thirteen each. The Graphiolaceae is represented by a single exotic species, *Graphiola phoenicis* Poit.

The book is concluded with an excellent reference section of over 600 selected references and a combination host and fungus index.

The species treatments are well annotated with interesting and helpful information on comparative morphology, spore germination, infection of the host and physiologic specialization wherever data on these biological phases are known.

The species concept employed by the authors is delightfully conservative, with the proper emphasis on morphological characters. Even my own cherished species consolidations in the grass and cereal smuts are employed. There are no new species and only three new combinations.

Illustrations consist of two full-page plates of half-tones illustrating macroscopic characters, and 21 text figures showing spores, germinating spores, and sporidia.

The book is as large as is justified by the 72 species recognized as occurring in the British Isles. It is well written, with profuse references to the literature. *The British Smut Fungi* will prove

a useful manual and reference work for mycologists, plant pathologists, and other botanists generally, as well as for students.—GEORGE W. FISCHER.

AUTOBIOGRAPHY, by W. A. Murrill. 165 pp. Gainesville, Fla. Published by the author. 1945. $2.00.

Following his 75th birthday celebration, at which he was feted by the University of Florida Chapter of The Society of the Sigma Xi as well as by many correspondents throughout the country, Dr. Murrill set down, at the request of some of his friends, reminiscences of some of the events of those 75 years of his life. The booklet in which these memories are presented is a 165 page paper-backed brochure including 28 chapters.

Throughout the account of his life, which is presented in chronological order, Dr. Murrill refers to himself as the "Naturalist." He was born in Lynchburg, Virginia, Oct. 13, 1869 and lived at Lynchburg until he was twelve when he was sent to the Virginia Agricultural and Mechanical College at Blacksburg from which he graduated in Agriculture; after another year he took a B.S. degree in Mechanics, then taught for a year in a small country school near Blacksburg.

From Randolph-Macon College in Ashland, Virginia, he received the first B.S. degree granted by that institution and later he received there an A.M. degree. At Blacksburg his courses were of a practical type, at Randolph-Macon they were literary and classical. His interest in literature and classics is reflected throughout the Autobiography in selected passages from English and American literature, and by occasional short essays concerning the natural scene as he saw it. In later life it was reflected in his scientific work by his style of writing and the facility with which he has been able to prepare Latin diagnoses for his specific and generic descriptions.

Following graduation from Randolph-Macon College the Naturalist taught botany and natural history to girls in such schools as Bowling Green Female Seminary and the Wesleyan Female Institute in Staunton, Virginia. At Staunton young Murrill finally decided to devote his life to some phase of natural history.

Murrill took a graduate appointment under Professors Atkinson and Comstock at Cornell University. That his botanical interests are wide is indicated by the title of his doctorate thesis "Fertilization in the Hemlock Spruce." At first he had temporary positions at Columbia University and the New York Botanical Garden. On Jan. 1, 1906, he assumed his permanent duties in the mycological herbarium at the Garden and on occasion served as acting director. He visited Europe several times in search of type material in herbaria for study in the preparation of his works on the polypores and other higher fungi. During this period he did spade work on the cause and possible cure of the chestnut blight.

He never lost sight of his interest in teaching natural history to young people. He was exceedingly active in popular lecture work and in girl scout work. His home was always open to the girl scouts and he spent quite a bit of time with them in camp in the eastern mountains.

During this period he was also able to make a number of extended collecting trips: to Jamaica, Washington, Oregon, California, Mexico, Maine, the Adirondacks and the Catskills. The hills of Virginia still lured him and he collected fungi on several trips there. On another occasion he made an extensive trip to South America from whence he brought back many specimens.

By August 1924 administrative duties became more pressing than Dr. Murrill liked and so he tendered his resignation. He retired to the home of an aunt in Lynchburg and near there he built himself a cabin in the woods where he lived for over a year. Following this hermit-like retirement he discovered Florida and especially the vicinity of Gainesville. There he built for himself a house near the University of Florida campus where he has lived most of the time since. At the Herbarium of the University he has been able to continue his mycological and natural history studies without the worry of administrative duties.

The book is written in a clear, easy style, and if it seems at times that there is too much of the attitude "God's in his heaven, all's well with the world," one must remember that for a long time Dr. Murrill has been able to identify himself with the natural scene in a way not permitted many of his readers.—WM. BRIDGE COOKE.

NOTES AND BRIEF ARTICLES

Campanella in Florida

A good supply of a fungus entirely new to me was collected by Mr. Daniel Roberts on a rotten magnolia log in a hammock near Gainesville, Fla., on April 25, 1948. Part of the collection was dried and Dr. Weber preserved the rest in fluid. Just as I was about to name it in honor of the collector, Dr. Singer published it in Lloydia **13**: 249. 1951. I'm glad he did, because he has done some good work on *Favolaschia* and *Campanella*, two of the hardest genera in the book.

Campanella floridana Singer was based on material he collected on an old magnolia log in a hammock at Newnan's Lake, east of Gainesville, July 31, 1947. This locality is about fifteen miles from where Roberts got his specimens. The species is probably the finest in the genus, with a pileus over an inch broad and a beautiful reticulated hymenium.—W. A. Murrill.

Western Fungi

Miss Elizabeth E. Morse, Hotel Claremont, Berkeley 5, California, is disposing of the balance of her collections. Those who would like to acquire specimens of western fleshy fungi by gift or exchange should communicate with her. Material received by exchange will be turned over to the herbarium of the University of California at Berkeley.

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